

00/82/TO

01-31-00



jc674 U.S. PTO

## TYPES, CROSS-NOTING, AND STATUS OF APPLICATION

201.06(a)

\*\*&gt; PTO/SB/13 (11-98)

Approved for use through 6/30/99. OMB 0651-0033  
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

## REQUEST FOR FILING A PATENT APPLICATION UNDER 37 CFR 1.60

DOCKET NUMBER	ANTICIPATED CLASSIFICATION OF THIS APPLICATION		PRIOR APPLICATION EXAMINER	ART UNIT
GEO 3.0-001 (DIV) (2)	CLASS	SUBCLASS	Examiner David Redding	1744

Address to:  
Assistant Commissioner for Patents  
Washington, D.C. 20231This is a request for filing a ☐ continuation ☒ divisional application under 37 CFR 1.60, of pending prior  
Application Number 08/862,782, filed on May 23, 1997, entitled ANAEROBIC  
BIOREMEDIATION SYSTEM

1. Enclosed is a copy of the latest inventor-signed prior application, including a copy of the oath or declaration showing the original signature or an indication it was signed. I hereby verify that the papers are a true copy of the latest signed prior application number 08/862,782 and further that all statements made herein of my own knowledge are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

CLAIMS	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
TOTAL CLAIMS (37 CFR 1.16(c))	6	- 20 =	--	x \$ 9 =	\$ --
INDEPENDENT CLAIMS (37 CFR 1.16(d))	1	- 3 =	--	x \$39 =	--
MULTIPLE DEPENDENT CLAIMS (if applicable) (37 CFR 1.16(e))				+ \$ =	--
BASIC FEE (37 CFR 1.16(a))					+ \$345.00
Total of above Calculations =					\$345.00
Reduction by 50% for filing by small entity (Note 37 CFR 1.9, 1.27, 1.28).					
TOTAL =					\$345.00

2. ☒ A verified statement to establish small entity status under 37 CFR 1.9 and 1.27  
☐ is enclosed.  
☒ was filed in prior application number 08/862,782 and such status is still proper and desired (37 CFR 1.28(a)).
3. ☐ The Commissioner is hereby authorized to charge any fees which may be required under 37 CFR 1.16 and 1.17, or credit any overpayment to Deposit Account No. \_\_\_\_\_. A duplicate copy of this sheet is enclosed.
4. ☒ A check in the amount of \$ 345.00 is enclosed.
5. ☒ Cancel in this application original claims 1 to 47 and 54 to 74 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)
6. ☒ The inventor(s) of the invention being claimed in this application is (are): ERIC CHRISTIAN HINCE, ROBERT L. ZIMMER, and TIMOTHY H. ANDERSON
7. ☐ This application is being filed by less than all the inventors named in the prior application. In accordance with 37 CFR 1.60(b), the Commissioner is requested to delete the name(s) of the following person or persons who are not inventors of the invention being claimed in this application:
8. ☒ Amend the specification by inserting before the first line the sentence: "This application is a ☐ continuation ☒ division of application number 08/862,782, filed May 23, 1997, (status, abandoned, pending, etc.)."
- allowed

[Page 1 of 2]

Burden Hour Statement: This form is estimated to take 0.5 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

jc675 U.S. PTO  
09/493579

01/28/00

\*\*&gt; PTO/SB/13 (11-96)

Approved for use through 6/30/99. OMB 0651-0033  
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

**(REQUEST FOR FILING A PATENT APPLICATION UNDER 37 CFR 1.60, PAGE 2)**

9. ☐ New formal drawings are enclosed.
10. ☐ Priority of foreign application number \_\_\_\_\_, filed on \_\_\_\_\_ in \_\_\_\_\_  
is claimed under 35 U.S.C. 119(a) - (d).  
☐ The certified copy has been filed in prior application number \_\_\_\_ / \_\_\_\_\_, filed \_\_\_\_\_.
11. ☒ A preliminary amendment is enclosed.
12. ☒ The prior application is assigned of record to GEOVATION CONSULTANTS, INC.
13. ☐ Also enclosed:
14. ☒ The power of attorney in the prior application is to: EZRA SUTTON, P.A.
- a. ☒ The power of attorney appears in the original papers in the prior application.
- b. ☐ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.
- c. ☐ Address all future correspondence to: (May only be completed by applicant, or attorney or agent of record.)

☐ Customer Number

OR

☒ Firm or☒ Individual Name

EZRA SUTTON, P.A.

Address Plaza 9, 900 Route 9

Address

City Woodbridge State NJ ZIP 07095

Country United States of America

Telephone 1-732-634-3520

Fax 1-732-634-3511

January 28, 2000

EZRA SUTTON, Reg. No. 25,770

Date

Signature

Typed or printed name

- ☐ Inventor(s)
- ☐ Assignee of complete interest. Certification under 37 CFR 3.73(b) is enclosed.
- ☒ Attorney or agent of record
- ☐ Filed under 37 CFR 1.34(a)  
Registration number if acting under 37 CFR 1.34(a) \_\_\_\_\_

Applicant or Patentee: ERIC CHRISTIAN HINCE Attorney's Docket  
Serial or Patent No.: \_\_\_\_\_ No. GEO 3.0-001  
Filed or Issued: \_\_\_\_\_  
Title: ANAEROBIC BIOREMEDIATION SYSTEM

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS  
(37 CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

- [ ] the owner of the small business concern identified below:  
[X] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN GEOVATION CONSULTANTS, INC.  
ADDRESS OF CONCERN 100 River Road, Triangle Plaza, Suite 8  
Harriman, New York 10926

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled: \_\_\_\_\_

ANAEROBIC BIOREMEDIATION SYSTEM  
by inventor(s) ERIC CHRISTIAN HINCE described in

- [X] the specification filed herewith  
[ ] application Serial No. \_\_\_\_\_, filed \_\_\_\_\_  
[ ] Patent No. \_\_\_\_\_, issued \_\_\_\_\_

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). \*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_  
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_  
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_  
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING ERIC CHRISTIAN HINCE  
TITLE OF PERSON OTHER THAN OWNER President  
ADDRESS OF PERSON SIGNING 100 River Road, Triangle Plaza, Suite 8  
Harriman, New York 10926  
SIGNATURE X [Signature] DATE 5/22/97

# SECRET

In re patent application of  
ERIC CHRISTIAN HINCE, et al

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

48. A solid chemical composition for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in contaminated geologic media, including one or more of the following granular Manganese (IV)-containing compositions, selected from the group consisting of glauconite, manganese greensand and pyrolusite.

49. A solid chemical composition in accordance with Claim 48 wherein the Manganese (IV) contained in said granular composition acts as an electron acceptor for manganese-reducing bacteria and/or other MRP anaerobic bacteria.

50. A solid chemical composition in accordance with Claim 49 wherein the composition is used as a component in a remediation trench, slurry-wall, semi-permeable barrier, as a well-packing material in a screened well, or other subsurface remedial application.

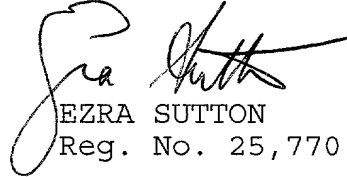
~~51.~~ A solid chemical composition for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in contaminated geologic media, comprised in whole or in part with minerals containing Fe (III) including Fe(III)OH and equivalents thereof.

52. A solid chemical composition in accordance with Claim 51 wherein the Fe (III) contained in said granular composition acts as an electron acceptor for iron-reducing bacteria and/or other MRP anaerobic bacteria.

53. A solid chemical composition in accordance with Claim 52 wherein the composition is used as a component in a remediation trench, slurry-wall, semi-permeable barrier, as a well-packing material in a screened well, or other subsurface remedial application.

Respectfully submitted,

EZRA SUTTON, P.A.



EZRA SUTTON  
Reg. No. 25,770

Plaza 9, 900 Route 9  
Woodbridge, New Jersey 07095  
(732) 634-3520

ES/jmt

Enclosures

ANAEROBIC BIOREMEDIATION SYSTEM

FIELD OF THE INVENTION

The present invention relates to a new and improved anaerobic  
bioremediation system and method thereof for anaerobic  
biodegradation, detoxification and transformation of toxic organic  
and inorganic compounds in contaminated geologic formations to  
harmless and safe organic and inorganic materials within the  
geologic media. More particularly, the bioremediation system  
includes a chemical composition and apparatus for implementing the  
conversion of contaminated organic and inorganic materials to  
clean, safe, and harmless materials via naturally occurring  
anaerobic bioremediation within the geologic media.

BACKGROUND OF THE INVENTION

Aerobic and anaerobic bioremediation of contaminated soils,  
ground water sites, lakes, ponds, aquifers, wells, shore fronts,  
oceans and the like have proven effective in remediating toxic  
organic and inorganic compounds such as fuel oil, gasoline, PCBs,  
DDT, and other pesticides, and the like. Bioremediation projects  
that have been successfully implemented are in areas that include  
underground storage tank spills and leakages; hazardous solid  
wastes; ground spills; and contamination of ground water (wells),  
geological aquifers and the like. Aerobic and anaerobic  
bioremediation have proven to be more cost effective and timely  
than conventional engineering technologies, and have the further  
advantage in that they do not produce wastes which enter the  
surrounding air, water and soils. Anaerobic bioremediation has been

shown to be the most effective and least expensive method of remediation of toxic materials. Other remediation technologies such as standard physical soil and ground-water remediation ie. excavation and disposal or pump and treat systems and soil-vapor extraction (SVE) remediation which have all been shown to be moderately high in capital costs; require long-term operation and maintenance including labor, materials and power consumption; time parameters that are in months to years; and remediation effectiveness results being moderately low in the cleaning-up of the contaminated soils and ground water. These remediation techniques are also limited by considerations of depth of soil to be removed; obstructions in the geologic formations; safety conditions at the site; and environmental law statutes with regard to Federal, State and local regulatory agencies for a site remediation project that produces wastes that effect air, water and soil conditions. These aforementioned remediation technologies generally are less feasible, and significantly more expensive and sometimes prohibitive in terms of logistics and/or overall costs.

Problems associated with anaerobic bioremediation have included the difficulty of achieving significant increases in the naturally occurring indigenous microorganisms at the contaminated site using appropriate chemical compositions of nutrients that would successfully have the indigenous anaerobic bacteria metabolize the organic and inorganic contaminants. The inability to maximize the rate of metabolism of toxic contaminants by anaerobic microorganisms can be due to inadequate or incorrect



electron acceptors, nutrient forms of nitrogen and phosphorus, trace-mineral micronutrients, chelating agents, non-toxic surfactants, lack of carbon co-substrates, and inerting agents. There is also a physical inability to deliver, distribute and  
5 disperse the nutrients readily, rapidly and over a wide distribution area within the contaminated geologic media for effective biodegradation of the contaminated substances into non-toxic end-products. Also, there is a difficulty of working with anaerobic microorganisms and processes because the biochemical  
10 pathways describing the anaerobic degradation of organic and inorganic compounds have been difficult to characterize, and to a large degree, are yet to be fully understood. Thus, it can be seen that anaerobic bioremediation of contaminated geologic media such as ground water (wells), sludge, soil and the like is not an easy or simple technological problem.

There remains a need for an improved method and apparatus for the anaerobic bioremediation of organic and inorganic toxic compounds within a contaminated geologic media to form non-toxic end products without further formation of waste by-products that  
20 effect air, water and soil qualities at the geologic site. In addition, there is a need that the bioremediation be accomplished by having an in-situ treatment of the contaminated geologic media such that the organic and inorganic contaminants are metabolized or transformed by naturally occurring indigenous, denitrifying and/or  
25 manganese-, iron- and sulfate-reducing anaerobic microorganisms using the aforementioned electron acceptors, in combination with

nutrients, surfactants, chelating agents, a diluent, and an inert gas to convert the contaminants within the contaminated geologic media into non-toxic end products. These naturally occurring indigenous microorganisms which operate using one or more respiration pathways are hereafter called "multiple respiration pathway" (MRP) microorganisms.

#### DESCRIPTION OF THE PRIOR ART

Methods, apparatus and chemical compositions having nutrients for bioremediation using anaerobic microorganisms have been disclosed in the prior art. For example, U.S. Patent No. 5,178,491 to Graves et al discloses a vapor-phase nutrient delivery system for in-situ bioremediation of soil. The nutrients are delivered in the vapor phase to the affected areas of the contaminated soil for utilization by microorganisms to promote the metabolism of organic contaminants by the microorganisms. The delivery system includes a series of pumps, nutrient tanks, conduits, and wells for implementing the bioremediation process. This prior art patent does not disclose the apparatus, process or chemical compositions for the bioremediation of contaminated geologic media as in the present invention.

U.S. Patent No. 5,265,674 to Fredrickson et al discloses an enhancement method of in-situ remediation of aquifers. This method of bioremediation is adapted to deliver microorganisms, enzymes, nutrients and electron donors to subsurface zones contaminated by nitrates in order to stimulate or enhance denitrification. The remediation system includes nutrient tanks, pumps, conduit/piping,

an injection well and reclamation tanks. This prior art patent does not disclose the apparatus, process or chemical compositions for the bioremediation of contaminated geologic media as in the present invention.

5 U.S. Patent No. 5,342,769 to Hunter et al discloses a method for bioremediation of liquid or slurry hazardous waste streams for the removal of halogenated hydrocarbons by using naturally occurring anaerobic microorganisms having methanogenic characteristics. The remediation method includes a series of  
10 reactors, pumps, vacuum pumps, and conduits. This prior art patent does not disclose the apparatus, process or chemical compositions for the bioremediation of contaminated geologic media as in the present invention.

15 U.S. Patent No. 5,384,048 to Hazen et al discloses an apparatus and method for in-situ bioremediation of contaminated ground water and/or contaminated subsurface soil by chlorinated hydrocarbons. A nutrient fluid is used to stimulate the growth and reproduction of indigenous aerobic microorganisms that are capable of degrading the contaminants. The apparatus used for the  
20 bioremediation process includes injection wells, pumps, conduits, and monitoring/sensor components. This prior art patent does not disclose the apparatus, process or chemical compositions for the bioremediation of contaminated geologic media as in the present invention.

U.S. Patent No. 5,398,756 to Brodsky et al discloses a process for the in-situ bioremediation of contaminated soil. The process includes the forming of at least one liquid permeable region within the contaminated soil region, introducing microorganisms, nutrients and the like for degrading contaminants in the contaminated soil region, and transmitting direct electric current through the contaminated soil region for degrading the contaminants by electrochemical means. This prior art patent does not disclose the apparatus, process or chemical compositions for the bioremediation of contaminated geologic media as in the present invention.

U.S. Patent Nos. 5,482,630 and 5,556,536 to Lee et al and Turk disclose a controlled denitrification process and system using a bacterial bed. This bioprocess system is used for the reduction of nitrate to nitrogen in a fluid medium, such that anaerobic bacteria fed by a carbon source are used for the nitrate reduction. A column of suspended beads are used as the anaerobic bacterial bed for denitrification. This prior art patent does not disclose the apparatus, process or chemical compositions for the bioremediation of contaminated geologic media as in the present invention.

U.S. Patent No. 5,560,737 to Schuring et al discloses a method and apparatus for pneumatic fracturing and multicomponent injection enhancement of in-situ bioremediation in treating subsurface soil contaminated with organic compounds. This bioremediation method and apparatus provides for reducing or eliminating non-naturally occurring, subsurface, liquid contaminants within a geologic formation, which involves the steps of pneumatically fracturing the

soil formation to produce a fracture network. A pressurized gas stream is used having nutrients, oxygen, electron acceptors, pH buffers, and possible bacterial augmentation to add a different culture of microorganisms to the soil formation when used in fracturing the soil formation. This provides the proper growth of the bioremediation microorganisms within the fracture network in which to degrade the contaminated materials within the water or soil formations. This prior art patent does not disclose the apparatus, process or chemical compositions for the bioremediation of contaminated geologic media as in the present invention.

None of the prior art patents teach or disclose the apparatus, process or chemical compositions for the bioremediation of contaminated geologic media as in the present invention. Also, none of the prior art patents teach or disclose the process or system of the current invention for the in-situ treatment of contaminated geologic media by using multiple-respiration pathway (MRP) anaerobic microorganisms such as denitrifying, manganese-, iron- and sulfate-reducing anaerobic microorganisms.

Accordingly, it is an object of the present invention to provide an anaerobic bioremediation system for the anaerobic biodegradation, detoxification and transformation of toxic organic and inorganic contaminants in contaminated geologic media into non-toxic compounds without further formation of regulated wastes or other undesirable by-products that effect the air, water and soil at environmental-contamination sites.

Another object of the present invention is to provide an anaerobic bioremediation system for in-situ treatment of geologic media containing organic and inorganic contaminants that are metabolizable or transformable by indigenous anaerobic bacteria capable of utilizing one or more multiple respiration pathways (MRP) including denitrification, manganese-reduction, iron-reduction and sulfate-reduction within the contaminated geologic media at a site.

Another object of the present invention is to provide an anaerobic bioremediation system having apparatus which enables the delivery of nutrients and electron acceptors having an inert carrier gas to the indigenous MRP anaerobic microorganisms in order to promote conditions favorable to the growth of these indigenous microorganisms such that the metabolism or transformation of the contaminants by these microorganisms can easily take place, without the use of implanted microorganisms at the contaminated site.

Another object of the present invention is to provide an anaerobic bioremediation system having apparatus which enables the nutrients and electron acceptors and an inert carrier gas to be more readily and rapidly dispersed in the contaminated geologic media and made more available to a large area within the contaminated geologic media.

Another object of the present invention is to provide an anaerobic bioremediation system having the nutrients, electron acceptors, chelating agents, surfactants and diluent with an inert carrier gas being in a chemical composition form that is readily

utilizable and metabolizable by the indigenous MRP anaerobic microorganisms.

Another object of the present invention is to provide an anaerobic bioremediation system that has the capacity to supply macro-nutrients, micro-nutrients, electron acceptors, surfactants, and chelating agents, as well as to modify the pH, redox potential and oxygen availability in the subsurface geologic media.

Another object of the present invention is to provide an anaerobic bioremediation system that has the capacity to supply organically usable phosphate to phosphate-limited environments whereby sodium hexametaphosphate or other forms of hydrolyzable ringed or linear polyphosphates are used as the primary source of phosphate as such compounds are much less prone to in-situ precipitation than other forms of phosphate which enables a major improvement for the stimulation and growth of indigenous MRP microorganisms in the contaminated geologic formation.

Another object of the present invention is to provide an anaerobic bioremediation system that has the capacity to supply nitrate as both an electron acceptor and the main source of assimilated (i.e., nutrient) nitrogen, taken together with other unique aspects of the chemical compositions of the present invention, which enables a major improvement for the stimulation and growth of indigenous MRP microorganisms in the contaminated geologic media.

Another object of the present invention is to provide an anaerobic bioremediation system having apparatus that is simple to construct and use and which enables efficient delivery and monitoring of the nutrients and electron acceptors for the optimum growth rate and kinetics of various indigenous, denitrifying and other MRP anaerobic microorganisms in order to maximize the rate of degradation and transformation of the contaminants into non-toxic compounds by these indigenous MRP microorganisms.

Another object of the present invention is to provide an anaerobic bioremediation system having a process that is inexpensive and easy to operate, especially in the field on a large scale basis.

Another object of the present invention is to provide an anaerobic bioremediation system having a process that can be performed rapidly and safely in the field and result in the site meeting environmental clean-up standards set by various governmental agencies more rapidly and at a lower cost than can be accomplished with other methods.

A further object of the present invention is to provide an improved anaerobic bioremediation system that can be easily produced in an automated and economical manner and is readily affordable by various responsible parties, consultants, contractors, engineers, governmental agencies and corporate users.



### SUMMARY OF THE INVENTION

In accordance with the present invention there are provided liquid chemical compositions for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in a contaminated geologic media under reducing conditions, including, but not limited to, denitrifying, manganese-reducing, iron-reducing and sulfate-reducing conditions. One such liquid chemical composition includes sodium nitrate in the range of one-fifth (0.2) to four (4) pounds per gallon of the chemical composition; sodium hexametaphosphate or other biologically hydrolyzable ring or linear polyphosphate in the range of one twentieth (0.05) to five (5) pounds per gallon of the chemical composition; a surfactant in the range of 0.01% to 10% by volume of the chemical composition; and a diluent in the form of water.

The present invention further includes a bioremediation apparatus for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in a contaminated geologic media. The bioremediation apparatus includes a first set of one or more storage tanks containing a chemical composition for anaerobic biodegradation of toxic compounds in contaminated geologic media; a plurality of quick disconnect valve couplings connected to the first set of storage tanks; at least one logic controller having a logic controller programmer component for opening and closing an automatic valve connected to the first set of storage tanks to supply the chemical composition to the contaminated geologic media; and a screened well connected to the first set of storage tanks for supplying the chemical composition to the contaminated geologic media.

The present invention also includes methods for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in

contaminated geologic media. The basic method includes pressurizing one or more storage tanks containing a chemical composition and an inert carrier gas; connecting a plurality of quick disconnect valve couplings to one or more pressurized storage tanks; connecting a well to an automatic ball valve for supplying the chemical composition and the inert carrier gas through the well to the contaminated geologic media; and opening and closing of the automatic ball valve to dispense the chemical composition and the inert carrier gas under pressure through the well to the contaminated geologic media. Methods are also disclosed for alternating the cycles of redox potential and the predominant microbial respiration pathway within the contaminated geological media.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Further objects, features, and advantages of the present invention will become apparent upon consideration of the detailed description of the presently-preferred embodiments, when taken in conjunction with the accompanying drawings wherein:

Figure 1 is a side elevational view of the anaerobic bioremediation system of the preferred embodiment of the present invention showing the bioremediation processing apparatus and its major component assemblies therein and in operational use;

Figure 2 is a front perspective view of the anaerobic bioremediation system of the preferred embodiment of the present invention showing the subsurface housing containing the bioremediation processing apparatus and its component parts contained therein; and in operational use. It is noted that there

is an alternate configuration for connecting the bioremediation processing apparatus as shown in the following two figures;

Figure 3A is a front perspective view of the anaerobic bioremediation system of the preferred embodiment of the present invention showing the dispensing apparatus assembly and its component parts contained therein and the electronic control assembly and its component parts contained therein. The product canisters are operated in series using this set-up.

Figure 3B is a front perspective view of the anaerobic bioremediation system of the preferred embodiment of the present invention showing an alternate dispensing apparatus assembly and its component parts contained therein and the electronic control assembly and its component parts contained therein. The product canisters are operated in parallel using this alternate set-up.

Although the other Figures show the dispensing apparatus assembly configured as shown in Figure 3A (series product canister operation), it should be noted that the system can also be set up and operated using the parallel product canister configuration depicted in Figure 3B;

Figure 4 is a front perspective view of the anaerobic bioremediation system of the preferred embodiment of the present invention showing the electronic control assembly and its component parts contained therein;

Figure 5 is a side elevational view of the anaerobic bioremediation system of the preferred embodiment of the present invention showing the bioremediation processing apparatus having

the dispensing apparatus assembly, the electronic control assembly, and the first well component assembly; and the vapor suppression system and a second well component assembly; with both systems being in operational use;

5        Figure 6 is a front perspective view of the anaerobic bioremediation system of the alternate embodiment of the present invention showing the vapor suppression system and its component parts contained thereon and in operational use;

10        Figure 7 is a front perspective view of the anaerobic bioremediation system of the preferred and alternate embodiments of the present invention showing dual dispensing apparatus assemblies and dual electronic control assemblies being electrically interconnected for the alternate feeding of the standard nutrient composition and alternative nutrient compositions into the well component assembly;

15        Figure 8 is a side elevational view of the anaerobic bioremediation system of the alternate embodiment of the present invention showing the bioremediation processing apparatus for use in bioremediation applications involving dense non-aqueous phase liquid contaminants (DNAPLs) having a dispensing apparatus assembly, an electronic control assembly and a well component assembly; and in operational use;

20        Figure 9 is a side elevational view of the anaerobic bioremediation system of the alternate embodiment of the present invention showing the bioremediation processing apparatus for use in bioremediation applications involving light non-aqueous phase

liquid contaminants (LNAPLs) having a dispensing apparatus assembly, an electronic control assembly and a well component assembly; and in operational use; and

Figure 10 is a schematic diagram of the anaerobic bioremediation system of the present invention showing the generic stoichiometric equations for the bioremediation processes of converting organic contaminants into non-toxic byproducts, such as carbon dioxide, nitrogen gas and water via denitrification.

Figure 11 is a schematic diagram showing a typical application of the use of the chemical compositions to facilitate the cycling of redox conditions and microbial respiration pathways in-situ beginning with denitrification followed by manganese-reduction, iron-reduction and sulfate-reduction and returning again to denitrification.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

### OVERVIEW OF THE INVENTION

The anaerobic bioremediation system, methods, chemical compositions and apparatus of this invention provide the means for the anaerobic biodegradation, detoxification and transformation of toxic organic and inorganic compounds in contaminated geologic media to harmless and safe organic and inorganic materials within the geologic media. In-situ bioremediation has recently emerged as the general category of site remediation technologies which provides for the most timely and effective remediation of contaminated soil and ground water from petroleum hydrocarbon spills, releases of halogenated hydrocarbons, solvents and

pesticides, inorganic chemical dumping, and the like. Furthermore, field demonstrations of bioremediation technologies have typically outperformed laboratory studies, even though it has often been assumed by experts in the field that ideal conditions were established in the laboratory. The success of bioremediation field trials, including those of the present invention, is thought to be attributable to the greater diversity of bacterial populations and their enzymatic processes that are present in the natural hydrogeologic settings versus those that can be established in laboratory microcosms.

In-situ bioremediation provides for the potential of a swift reduction of contaminant levels, often in periods as short as weeks to months, as shown in examples of the actual use of the present invention on contaminated geologic formations as provided below. The present invention uses naturally-occurring bacteria that are indigenous to the geologic formation being remediated for the degradation of hydrocarbons, solvents, pesticides, hazardous wastes and the like. The theoretical basis and effectiveness of using indigenous MRP anaerobic microorganisms capable of denitrification, manganese-reduction, iron-reduction and sulfate-reduction is demonstrated in the bioremediation process diagram (Figure 10) which describes the theoretical operation of the bioremediation system 10 of the present invention. The present invention provides a means of stimulating such MRP anaerobic microorganisms so as to achieve rapid and effective degradation and remediation of aromatic hydrocarbons as well as halogenated hydrocarbons, pesticides,

hazardous wastes and other contaminants as demonstrated in the forthcoming examples of projects conducted at actual contaminated geologic sites.

The principle of using anaerobic microorganisms such as denitrifying bacteria and other MRP anaerobic bacteria in the present invention is dependent upon the natural sequence of electron-acceptor utilization by bacteria within geologic media as well as the natural occurrence and/or solubility of these electron acceptors in water. Bacteria utilize electron acceptors in the order of their decreasing energy yield (Gibbs Free Energy [ $\Delta G$ ] in KJ/mole  $\text{CH}_2\text{O}$ ). In theory, as the availability of a higher-energy electron acceptor wanes (e.g.,  $\text{O}_2$ ), conditions become favorable for microbial respiration with lower-energy electron acceptors (e.g.,  $\text{NO}_3$ ,  $\text{Mn(IV)}$ ,  $\text{Fe(III)}$ , and  $\text{SO}_4$ ). The natural sequence of microbial utilization of electron acceptors in the environment is summarized in Table 1 below:

TABLE 1 Energy Yield and Solubility of Microbial Electron Acceptors		
Electron Acceptor	[ $\Delta G$ ] in KJ/mole $\text{CH}_2\text{O}$ <sup>a</sup>	Max. Solubility (mg/l) <sup>b</sup>
$\text{O}_2$	-475	$10^c$
$\text{NO}_3$	-448	637,000 <sup>d</sup>
$\text{MnO}_2$	-349	Insoluble mineral
$\text{Fe(OH)}_3$	-114	Insoluble mineral
$\text{SO}_4$	-77	113,000 <sup>e</sup>
$\text{CO}_2$	-58	2,000 <sup>f</sup>

<sup>a</sup>Based on unpublished data from Robert C. Aller (1987), Marine Sciences Research Center, The State University of New York at Stony Brook.

<sup>b</sup>Unless otherwise specified, solubility is that in water under typical "water table" conditions: 15°C, 1 atm. pressure.

<sup>c</sup>American Public Health Association et al. (1992).

<sup>d</sup>Solubility of NO<sub>3</sub> as a solution of sodium nitrate (NaNO<sub>3</sub>).

<sup>e</sup>Solubility of SO<sub>4</sub> as a solution of ferrous sulfate heptahydrate at 0°C. (Solubility is 513,323 at 50°C).

<sup>f</sup>Solubility of CO<sub>2</sub> (as CO<sub>2</sub>) at 15°C estimated from Perry's Chemical Engineers' Handbook, Sixth Edition, 1984.

Note: The natural sequence of microbial electron-acceptor utilization described in Table 1 is based on the original research of Froelich et al. (1979).

Most bioremediation processes described in the prior art and in the literature have involved the addition of oxygen in attempts to facilitate the aerobic degradation of various organic contaminants. However, it is very difficult to maintain sufficient concentrations of oxygen in-situ to support aerobic biodegradation, largely due to the low solubility of oxygen in ground water ( $\leq 10$  mg/l), as well as the multitude of both biological and chemical "sinks" of oxygen in the subsurface. In contaminated ground waters, redox conditions are typically strongly reducing and dissolved oxygen concentrations are typically insufficient (i.e.,  $\leq 1$  mg/l) to support strictly-aerobic respiration. Therefore, given the low solubility and reactivity of oxygen, it is typically



impractical to maintain non-limiting conditions of oxygen-availability in the subsurface.

As shown in Table 1 above, nitrate and sulfate salts are much more soluble in water than is oxygen. Nitrate and sulfate are also more "conservative" than oxygen in terms of their geochemistry, i.e., these species are less reactive and more mobile. Therefore, diffusive processes can be used to deliver non-limiting concentrations of nitrate and sulfate to the interior of groundwater contaminant plumes in relatively short periods of time because of the significant in-situ concentration gradients that can be established by the present invention.

Although aeration of subsurface sediments (e.g., air sparging) has been used with some success, a documented problem with the aeration and/or oxygenation of contaminated ground waters containing elevated concentrations of iron and manganese (i.e.,  $\geq 5$  mg/l) is that such processes lead to the oxidation and precipitation of these species in-situ. Accordingly, aeration, oxygen-injection, peroxide injection and other similar aerobic bioremediation processes tend to reduce the effective porosity and hydraulic conductivity of the geologic media in-situ which ultimately slows the rate of site remediation. Iron oxidation and fouling problems have often been encountered in pump and treat systems as evidenced by air-strippers which have become fouled with iron. The use of the present invention however, avoids this problem altogether by focusing on the enhancement of the naturally anaerobic and reducing conditions present in contaminated geologic

media.

As shown in Table 1 above, nitrate provides denitrifying bacteria with a significant Gibbs-Free-Energy ( $\Delta G = -448$  KJ/mole  $\text{CH}_2\text{O}$ ), which is within approximately 5% of that of oxygen. In addition, denitrification is more efficient (if not more rapid) than aerobic processes, as only 1 mole of nitrate versus 1.25 moles of oxygen is consumed in the degradation of one mole of contaminant. Reactions which describe aerobic, denitrification, manganese-reduction, iron-reduction, and sulfate-reduction mediated biodegradation of an idealized hydrocarbon contaminant ( $-\text{CH}-$ ) are provided in Table 2 below:

TABLE 2	
Bioremediation Respiration Pathway	Generalized Stoichiometric Equation
(1) Aerobic	$12-\text{CH}- + 15\text{O}_2 \rightarrow 12\text{CO}_2 + 6\text{H}_2\text{O}$
(2) Denitrification	$-\text{CH}- + \text{NO}_3 + \text{H}^+ \rightarrow \text{CO}_2 + 1/2\text{N}_2 + \text{H}_2\text{O}$
(3) Manganese Reduction	$-\text{CH}- + \frac{1}{2} \text{Mn (IV)} + 3\text{H}_2\text{O} \rightarrow \frac{1}{2} \text{Mn(II)} + \text{HCO}_3^- + 6\text{H}^+$
(4) Iron Reduction	$-\text{CH}- + \text{Fe(III)} + 3\text{H}_2\text{O} \rightarrow \text{Fe(II)} + \text{HCO}_3^- + 6\text{H}^+$
(5) Sulfate Reduction	$-\text{CH}- + \text{SO}_4^{-2} + 5\text{H}^+ \rightarrow \text{CO}_2 + \text{H}_2\text{S} + 2\text{H}_2\text{O}$

As shown in Table 2 above, the ultimate end-products of denitrification are carbon dioxide, water and elemental-nitrogen gas. Consequently, the present invention facilitates the use of denitrification as a naturally safe and practical means of

bioremediation as shown in the bioremediation process 10 of the present invention. When combined with the stimulation of manganese-reducing, iron-reducing and sulfate-reducing bacteria as enabled by this invention, a significant and consistently demonstrable improvement in site remediation is provided.

#### OVERVIEW OF THE BIOREMEDIATION CHEMICAL COMPOSITIONS

With regard to the present invention, naturally occurring (i.e., indigenous) microorganisms that are capable of using one or more multiple respiration pathways are hereafter defined as multiple respiration pathway (MRP) microorganisms. The use of the present invention provides a means for the stimulation and maintenance of either denitrifying conditions alone or, alternatively, a means of alternating and/or cycling through a natural range of oxidation-reduction potentials and the related sequence of anaerobic microbial respiration pathways including denitrification, manganese-reduction, iron-reduction and sulfate-reduction. The bioremediation processes of the present invention are represented by the general stoichiometric equations shown in Figure 10 of the drawings and in Table 2 above. The present invention enables these processes to occur either independently, in succession or in different zones within the contaminated geologic media.

The bioremediation nutrient fluids of the present invention are chemical compositions for use in the anaerobic bioremediation system 10 of the present invention, and are essential and extremely important factors for stimulating the growth and reproduction of

the indigenous MRP anaerobic microorganisms 12 which are capable of degrading the organic contaminants 14 into safe and harmless end products such as carbon-dioxide gas (CO<sub>2</sub>) 16, nitrogen gas (N<sub>2</sub>) 18 and water (H<sub>2</sub>O) 20 and various salts and organic acids depending on both the contaminant of concern and the anaerobic respiration process involved. The chemical compositions of the present invention include electron-acceptor compounds for stimulating microbial respiration, macro-nutrients for providing substrates for biosynthesis and growth, a diluent, chelating agents, a biodegradable surfactant agent for increasing the biological availability of organic contaminants and controlling the surface activity of the nutrient chemical compositions into and throughout the contaminated geologic media, and an inert carrier gas 22 for providing anaerobic conditions (i.e., no oxygen) for maximizing the growth of the MRP anaerobic bacteria 12 used within the bioremediation system 10, as well as to maintain either the gravitational or passive flow of the inert carrier gas 22 into the well or the addition of these materials under pressure. Electron donors are typically excluded from the chemical compositions but can be provided as either co-substrates or primary substrates in the chemical compositions of the present invention to help the MRP microorganisms either co-metabolize organic contaminants or biotransform inorganic contaminants, respectively. Similarly, micronutrients are typically excluded from the chemical compositions as the naturally-occurring trace metal micronutrients found in the geologic media at most contaminated sites typically

provide an abundant supply of micronutrients; nonetheless, the present invention provides for a means of delivering micronutrients and/or chelating agents (to enhance micronutrient availability and transfer) to the contaminated geologic media.

5 CHEMICAL COMPOSITION 40

10 The present invention includes a first chemical composition 40 for maximizing and facilitating contaminant degradation by indigenous MRP anaerobic microorganisms 12 within the contaminated geologic media. The preferred embodiment of the chemical nutrient fluid composition 40 includes a primary compound acting as both an electron acceptor and nutrient being sodium nitrate in the range of 0.2 to four (4) pounds per gallon of the chemical composition (2% to 32% by weight of the diluent), and a second nutrient compound being a biologically usable phosphate in the form of sodium hexametaphosphate or other biologically hydrolyzable ring or linear polyphosphate in the range of one twentieth (0.05) to five (5) pounds per gallon of the chemical composition (0.1% to 11% by weight of the diluent). The molar ratios of nitrate ( $\text{NO}_3^-$ ) to phosphorus (P) are preferably in the range of 2:1 to 50:1, although site specific conditions may require deviations from this range.

20 The chemical composition 40 further includes biodegradable, non-toxic, surfactant agents such as Biosolve™ and equivalents thereof in the range of 0.01% to 10% by volume.

25 The chemical nutrient fluid composition 40 may further include chelating agents including citric acid, sodium citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid

(EDTA), and equivalents thereof; in the range of 0.05 to 3 pounds per gallon of the chemical composition (0.1% to 6% by weight of the diluent).

These compounds are combined in the proper order with a suitable diluent, such as water.

The carrier gas 22 used in conjunction with the chemical composition 40 includes inert gases such as argon, neon, krypton, or xenon.

#### CHEMICAL COMPOSITION 41

The present invention further includes a first alternate embodiment 41 for maximizing and facilitating contaminant degradation by indigenous MRP anaerobic microorganisms 12 within the contaminated geologic media. Chemical nutrient composition 41 substitutes all or some of the sodium nitrate characterized in chemical composition 40 with a primary compound acting as both electron acceptor and nutrient being dissolved nitrous oxide ( $N_2O$ ) in the range of one one hundredth (0.01) to one fiftieth (0.02) pounds per gallon of the chemical composition (1% to 2% by weight of the diluent), and a second nutrient compound being a biologically usable phosphate in the form of sodium hexametaphosphate or other biologically hydrolyzable ring or linear polyphosphate in the range of one two hundredth (0.005) to five (5) pounds per gallon of the chemical composition (0.01% to 11% by weight of the diluent). The molar ratios of the combined nitrous oxide and nitrate nitrogen (N) to phosphorus (P) are preferably in the range of 0.5:1 to 50:1, although site specific conditions may

require deviations from this range.

The chemical composition 41 further includes biodegradable, non-toxic, surfactant agents such as Biosolve™ and equivalents thereof in the range of 0.01% to 10% by volume.

5       The chemical nutrient fluid composition 41 may further include chelating agents including citric acid, sodium citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), and equivalents thereof; in the range of 0.05 to 3 pounds per gallon of the chemical composition 41 (0.1% to 6% by weight of  
10       the diluent).

These constituents are combined in the proper order with a suitable diluent, such as water.

15       The carrier gas 22 used in conjunction with the chemical composition 41 includes inert gases such as argon, neon, krypton, or xenon. In addition, some or all of the inert gas may be replaced with gaseous phase electron acceptors such as nitrous oxide or equivalent. Additionally, the gaseous phase electron acceptors such as nitrous oxide or equivalent chemicals may be used for direct infiltration into the contaminated geologic media to  
20       facilitate bioremediation of said media.

#### CHEMICAL COMPOSITION 42

25       The present invention also includes a second alternate embodiment 42 for maximizing and facilitating contaminant degradation by indigenous sulfate-reducing and related MRP anaerobic microorganisms 12 within the contaminated geologic media. Chemical nutrient composition 42 includes a primary compound acting

as an electron acceptor being ferrous sulfate heptahydrate, sodium sulfate or other soluble salts of sulfate in the range of one-half (0.5) to four (4) pounds per gallon of the chemical composition (approximately 1% to 25% by weight of the diluent), and a nutrient compound being a biologically usable phosphate in the form of sodium hexametaphosphate or other biologically hydrolyzable ring or linear polyphosphate in the range of one twentieth (0.05) to five (5) pounds per gallon of the chemical composition (0.1% to 11% by weight of the diluent). The molar ratios of the sulfate ( $\text{SO}_4^{2-}$ ) to phosphorus (P) are preferably in the range of 2:1 to 50:1, although site specific conditions may require deviations from this range.

The chemical composition 42 further includes biodegradable, non-toxic, surfactant agents such as Biosolve™ and equivalents thereof in the range of 0.01% to 10% by volume.

The chemical nutrient fluid composition 42 may further include chelating agents including citric acid, sodium citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), and equivalents thereof; in the range of 0.05 to 3 pounds per gallon of the chemical composition (0.1% to 6% by weight of the diluent).

These constituents are combined in the proper order with a suitable diluent, such as water.

The carrier gas 22 used in conjunction with the chemical composition 42 includes inert gases such as argon, neon, krypton, or xenon.



15

5

20

25

per gallon of the chemical composition (0.1% to 6% by weight of the diluent).

These constituents are combined in the proper order with a suitable diluent, such as water.

5        The carrier gas 22 used in conjunction with the chemical composition 43 includes inert gases such as argon, neon, krypton, or xenon.

#### CHEMICAL COMPOSITION 44

10        The present invention also includes a fourth alternate embodiment 44 for maximizing and facilitating contaminant degradation by indigenous anaerobic microorganisms 12 within the contaminated geologic media. Chemical nutrient composition 44 includes a primary compound acting as a source of both electron acceptor nitrogen and both nutrient nitrogen and micronutrient manganese being manganese nitrate, manganese nitrate tetrahydrate, or manganese nitrate hexahydrate in the range of one (1) to ten (10) pounds per gallon of the chemical composition (2% to 19% by weight of the diluent), and a nutrient compound being a biologically usable phosphate in the form of sodium hexametaphosphate or other biologically hydrolyzable ring or linear polyphosphate in the range of one twentieth (0.05) to five (5) pounds per gallon of the chemical composition (0.1% to 11% by weight of the diluent). The molar ratios of the combined nitrate and manganese to phosphorus (P) are preferably in the range of 2:1 to 50:1, although site specific conditions may require deviations from this range.

The chemical composition 44 further includes biodegradable, non-toxic, surfactant agents such as Biosolve™ and equivalents thereof in the range of 0.01% to 10% by volume.

The chemical nutrient fluid composition 44 may further include chelating agents including citric acid, sodium citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), and equivalents thereof; in the range of 0.05 to 3 pounds per gallon of the chemical composition (0.1% to 6% by weight of the diluent).

These constituents are combined in the proper order with a suitable diluent, such as water.

The carrier gas 22 used in conjunction with the chemical composition 44 includes inert gases such as argon, neon, krypton, or xenon.

#### CHEMICAL COMPOSITION 45

The present invention also includes a fifth alternate embodiment 45 for maximizing and facilitating contaminant degradation by indigenous anaerobic microorganisms 12 within the contaminated geologic media. Chemical nutrient composition 45 contains no added compound acting as a source of electron acceptor. The primary ingredient of alternate embodiment 45 is a chelating agent which makes transition metals present in the geologic media more biologically available to MRP, with this chelating agent being citric acid, sodium citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), or equivalents thereof; in the range of 0.05 to 3 pounds per gallon of the chemical

composition (0.1% to 6% by weight of the diluent), although site-specific conditions may require deviations from this range. Chemical nutrient composition 45 also contains a nutrient compound being a biologically usable phosphate in the form of sodium hexametaphosphate or other biologically hydrolyzable ring or linear polyphosphate in the range of one twentieth (0.05) to five (5) pounds per gallon of the chemical composition (0.1% to 11% by weight of the diluent), although site specific conditions may require deviations from this range.

The chemical composition 45 further includes biodegradable, non-toxic, surfactant agents such as Biosolve™ and equivalents thereof in the range of 0.01% to 10% by volume.

The carrier gas 22 used in conjunction with the chemical composition 45 includes inert gases such as argon, neon, krypton, or xenon.

#### SOLID CHEMICAL COMPOSITIONS 46

In addition, the present invention can also use solid chemical compounds such as glauconite, manganese greensand (a processed form of glauconite enriched in Mn(IV)), or pyrolusite, a mineral with the chemical formula of  $Mn(IV)O_2$ , where the Mn(IV) acts as an electron acceptor for manganese-reducing bacteria and/or MRP anaerobic bacteria. The glauconite, manganese greensand and/or pyrolusite can be used as a component of the well packing materials in combination with the filter sand for the well component assembly 150. The glauconite, manganese greensand and/or pyrolusite can also be used as a component in a remediation trench, slurry-wall,

semi-permeable barrier or other subsurface remedial applications.

In addition, the present invention can also use other solid chemical compounds comprised in whole or in part with minerals containing Fe(III), such as Fe(III)OH. The Fe(III) acts as an electron acceptor for iron-reducing bacteria and/or other MRP anaerobic bacteria. The Fe(III) containing materials can be used as a component of the well packing materials in combination with the filter sand for the well component assemblies 150. The Fe(III) containing materials can also be used as a component in a remediation trench, slurry-wall, semi-permeable barrier or other subsurface remedial applications

#### OPERATION OF THE COMPOSITIONS

The use of chemical compositions 40 through 46 in the present invention provides a means for stimulating various redox conditions and microbial respiration pathways *in-situ* within the contaminated geologic media to enhance the growth of MRP anaerobic microorganisms and to optimize contaminant biodegradation and/or biotransformation by such microorganisms. In addition, use of chemical compositions 40 through 46 in the present invention provide a means for cycling through a series of redox conditions *in-situ* within the contaminated geologic media as illustrated in Figure 10. Redox cycling within the contaminated geologic media *in-situ* is achieved by using one or more chemical compositions 40 through 46 in such a manner so as to stimulate a temporal and/or spatial succession of redox conditions and anaerobic respiration pathways in the subsurface. A typical application of the use of

chemical compositions 40 through 46 facilitates redox cycling beginning with denitrification followed by manganese-reduction, iron-reduction and sulfate-reduction and returning again to nitrate reduction as shown on Figure 11. For example, chemical compositions 40, 41, 43 and/or 44 are used to stimulate denitrification, followed by the use of chemical compositions 43, 44, 45 and/or 46 to stimulate manganese-reduction, iron-reduction and/or the reduction of other metals which can serve as microbial electron acceptors, followed by the use of chemical composition 42 to promote the growth of anaerobic bacteria via sulfate-reduction, followed by the use of chemical compositions 40, 41 and/or 43 to return again to denitrifying conditions. Because of the nature of the chemical compositions, methods and apparatus of the present invention, this invention also provides a means for varying such redox cycles to meet site-specific conditions or otherwise difficult contamination problems. The aforementioned cycling of redox conditions as facilitated by the present invention provides for the stimulation of a much more diverse community of MRP anaerobic microorganisms than could otherwise be achieved by other methods, which in turn provides a means of optimizing contaminant biodegradation and/or biotransformation *in-situ* within contaminated geologic media.

In addition, the use of chemical compositions 40 through 46 provides a means for providing MRP anaerobic bacteria with macro-nutrients and micronutrients needed to sustain bacterial growth and to promote biodegradation and/or biotransformation of organic and

inorganic contaminants. For example, in addition to nitrate and/or other anaerobic electron acceptors, bacteria also require macro-nutrients such as inorganic nitrogen (e.g., ammonium) and phosphate. Bacteria utilize ammonium and similar forms of nitrogen to help synthesize proteins and other complex organic molecules. However, excessive ammonium concentrations can inhibit denitrifying bacteria such as certain *Pseudomonas* spp., and other MRP anaerobic bacteria. Moreover, research related to the present invention has shown that ammonium is produced in adequate quantities for biosynthesis as a result of dissimilatory nitrate reduction by *Geobacter* spp. and other MRP anaerobic microorganisms. Accordingly, the present invention minimizes or eliminates ammonium addition, allowing native anaerobic microorganisms to produce and/or recycle ammonium from microbiological wastes.

Bacteria also require phosphate for the production of nucleic acids, phospholipids, and other biochemicals as well as for the maintenance of adequate levels of nucleoside 5' triphosphates such as adenosine-triphosphate (ATP), the most common intracellular "energy-molecule." Some researchers have shown that the availability of 10-20 mg/l of phosphate ( $\text{PO}_4^{3-}$ ) is typically sufficient to stimulate the biodegradation of aromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylenes (BTEX) in ground water. However, most phosphate salts cause precipitates to form close to the injection well screens because of the reactive geochemistry between phosphate and the cations naturally present in geologic media. Accordingly, the present invention uses sodium

hexametaphosphate and/or other ringed or linear polyphosphates for phosphate addition which helps overcome the typical fouling problems encountered by using other forms of phosphate. In addition, this invention provides a means of providing chelating agents to minimize abiotic reactions between phosphate and the naturally occurring cations in the geologic media.

Trace metal micronutrients, including, but not limited to, iron, molybdenum, copper, cobalt, manganese, boron and zinc are also important to the growth of denitrifying bacteria and other MRP anaerobic bacteria. These trace metals are required in the key enzymatic processes by which nitrate-reducing bacteria and other MRP anaerobes metabolize carbon sources such as hydrocarbons, halogenated solvents, pesticides, hazardous wastes and the like. For example, previous research reported in the literature has indicated that the addition of  $\geq 10 \mu\text{g}/\ell$  of key trace metals along with nitrate and phosphate facilitated the more effective degradation of BTEX compounds relative to the addition of nitrate and phosphate alone. Although these micronutrients are important to the growth of MRP anaerobic bacteria, research related to the operational use of the present invention, including experience in various geologic settings and contaminant conditions, has shown that the soil and ground water at each of these sites provides an adequate supply of these micronutrients. Therefore, the bioremediation system 10 of the present invention and the applications thereof minimize or eliminate the need for micronutrient addition, as the interaction of the chemical



compositions 40 through 46 with the naturally reducing and anaerobic conditions at typical contamination sites enhances micronutrient availability and transfer *in-situ* within the contaminated geologic media.

5           The substrate which is used as an electron donor within the contaminated geologic formation for anaerobic biodegradation could include organic chemical compounds or contaminants including petroleum-based hydrocarbons, halogenated hydrocarbons and solvents, polychlorinated biphenyls (PCB's), dioxin, pesticides, and other toxic/hazardous wastes. Examples of typical petroleum hydrocarbons include gasoline, diesel fuel, fuel oils and lubricating oils, as well as gasoline and diesel additives such as methyl tertiary butyl ether (MTBE), ethanol, tertiary butyl alcohol (TBA) and the like. Examples of typical halogenated hydrocarbons and solvents that are used as a carbon source by MRP anaerobic bacteria during the remediation of a contaminated site could include carbon tetrachloride, tetrachloroethylene, tetrachloroethane, trichloroethylene, 1,1,1,-trichloroethane, 1,1,2-trichloroethane, 1,2-dichloroethylene, chloroform, methylene chloride, 1,2-dibromoethane, 1,2-dichloroethane, vinyl chloride, trichlorofluoromethane (Freon 113), and the like. Typical pesticides, herbicides, insecticides, miticides, and nitroaromatic compounds being remediated at a contaminated site could include dinoseb (2-(1-methylpropyl)-4,6-dinitrophenol, DDT, DDD, DDE, Diazanon™, chlordane, malathion, trinitrotoluene (TNT), dinitrotoluene (DNT), toxaphene, and the like. Typical inorganic

contaminants and/or hazardous-wastes being remediated could include cyanides, cobalt-60, hexavalent chromium, uranium (VI), and other transition metals with the potential for reduction from higher valence states to lower valence states.

5

#### BIOREMEDIATION PROCESSING APPARATUS 50

The bioremediation processing apparatus 50 for the anaerobic bioremediation system 10 of the preferred embodiment of the present invention is represented in Figures 1 through 4 of the drawings. The bioremediation processing apparatus 50 is the delivery and feeding mechanism for transporting the nutrient fluid chemical composition 40 to the contaminated geologic formation 30 in order to stimulate anaerobic biodegradation, detoxification and transformation of toxic organic and inorganic compounds into harmless and safe end-products.

10

15

The bioremediation processing apparatus 50, as shown in Figures 1 to 3, includes a metal or concrete housing component 52, a dispensing apparatus assembly 80 for dispensing of chemical composition 40; an electronic control assembly 130 for electronically metering the chemical nutrient composition 40; and a well component assembly 150 for delivering of chemical nutrient composition 40 to the contaminated geologic formation 30. Bioremediation processing apparatus 50 includes a cylindrical subsurface housing 52 made of metal or concrete having an outer cylindrical bentonite seal layer 54. Housing 52 also includes an outer manhole cover 58 having an insulation layer 60 attached to the cover inner wall member 62. In addition, sub-surface housing

20

25

52 includes a cylindrical wall member 64 having inner and outer surface walls 66 and 68, and a bottom wall member 70 being a gravel layer. Bottom gravel wall member 70 further includes a top surface wall 72 and a bottom surface wall 74. Bottom wall member 70 includes a circular hole opening 76 within the gravel layer for receiving the upper end 154 of well casing 152. Insulation layer 60 on cover 58 protects the nutrient chemical composition 40 within the plurality of product canister tanks 100a to 100f of dispensing apparatus assembly 80 from freezing.

The dispensing apparatus assembly 80, as shown in detail by Figures 1, 2, and 3A of the drawings, includes a carrier gas cylinder 82 for holding of inert gas 22 having a gas regulator assembly 84, a plurality of stainless steel product canister tanks 100a to 100f for holding of the chemical nutrient composition 40 therein having removable lids 102a to 102f, and a plurality of quick disconnect couplings 104a to 104j for tanks 100a to 100f. A plurality of jumper line tubing 106a to 106f is attached to the aforementioned couplings 104a to 104j for connecting each of the product canister tanks 100a to 100f in series. In addition, the plurality of quick disconnect couplings 104a to 104j and the plurality of jumper line tubing 106a to 106f use a plurality of hose barb adapters 108a to 108j and 110 and a plurality of stainless steel hose clamps 112a to 112j for connecting the aforementioned quick disconnect couplings 104a to 104j and jumper line tubing 106a to 106f with each other.



the contaminated geologic formation 30 at precise time intervals. In addition, logic controller 132 is powered by a battery pack 142 via electrical lines 140 for operating in the field where no electrical power outlets are available. It is noted that battery pack 142 and electrical line 140 can be contained within the logic controller 132, as well as externally as shown in the drawings. Chemical nutrient composition 40 is discharged through the automatic ball valve 138 when it is in the open position, into a length of discharge tubing 148, as shown in Figures 2 through 5.

Logic controller 132 further includes as an metering of the chemical composition 40 to the contaminated geologic formation 30, as an option a plurality of digital and/or fiber-optic sensors 144a to 144i for the in-situ monitoring of the static-water levels, the changes in static-water levels, the in-situ concentrations of each of the components of the chemical compositions or the by-products thereof, the rate of use of one or more of the components of the chemical compositions by the MRP microorganisms, the total estimated mass of the microorganisms in-situ, the biomass growth rate of the naturally occurring MRP microorganisms in-situ, the conversion rates of the converted end-products being generated by the MRP microorganisms, the pH and/or redox potential of the saturated geologic media or biomass, and the temperature of the saturated geologic media and other pertinent measurable data needed. Logic controller 132 also includes a display component 146 for displaying the sensor outputs; as well as a telecommunication data link and telemetry phone lines 141 and 143, respectively, for

communicating the controller data to and receiving program changes from an off-site location.

Bioremediation processing apparatus 50 further includes an optional pressurized water feed 109, as shown in Figure 1. The pressurized water feed consists of a pressurized water line 111, connected to a pressurized water supply main; a pressure-reducing valve 113; a logic controller 132'; a manually-operated ball valve a 114'; and discharge tubing 115. The purpose of the optional pressurized water feed is to (1) provide additional fluid to periodically flush the concentrated fluid composition through the contaminated geologic media 30 at precise rates and time intervals, and (2) to increase saturation of the contaminated geologic media.

The well component assembly 150, as shown in Figure 1, includes a PVC well riser 152 having an upper end section 154 and a lower end PVC screen section 156 having slotted openings 158 within. The well PVC riser 152 is surrounded by backfilled soil cuttings 160 and a bentonite seal 162 at the upper end section 154 of the well PVC riser 152; and is surrounded by a Morie (or equivalent) sand pack 164 at the lower end section 156 of the well PVC riser 152. In addition, the well PVC riser 152 includes an inlet opening 166, a plurality of discharge outlet openings 168s and a bottom end cap 169. The chemical nutrient composition 40 is discharged into the contaminated geologic media 30 through discharge openings 168s via the PVC screen 156 of well 152.

The use of well 152 installed within a contaminated geologic formation 30 is to form an interface area 170 for bioremediation

000210-012600  
15555400

applications including light non-aqueous phase liquid contaminants (LNAPLs) where these contaminants have a specific gravity of less than one, as shown in Figure 9, and/or installed to form an interface area 170 with one or more hydrogeologic aquitards 36 for the bioremediation applications involving dense non-aqueous phase liquid contaminants (DNAPLs) where these contaminants have a specific gravity of more than one, as shown in Figure 8. The use of the PVC screen 156 within well 152 at depths of no more than 0.5 ft. to 10 ft. below the seasonal low of the water table level 28, as shown in Figure 9 of the drawings, is for the bioremediation applications involving light non-aqueous phase liquid contaminants (LNAPLs) where these contaminants have a specific gravity of less than one.

Outlet tubing 148 connected to the manual ball valve 114 extends into the inlet opening 166, as shown in Figure 1, for discharging of chemical nutrient composition 40 into the contaminated geological formation 30 through multiple outlet opening 168s.

#### SUBSURFACE VAPOR INERTING SYSTEM 200

The present invention further includes an improved and optional subsurface vapor-inerting system 200, as depicted in detail by Figures 5 and 6 of the drawings. The subsurface vapor-inerting system 200 is used for the reduction of oxygen gas ( $O_2$ ) concentrations 24 within the vadose zone section 38 of the contaminated geologic site 30 which provides fire safety prevention that reduces and/or eliminates flash fires and/or explosion hazards

associated with oxygen gas 24 and hydrocarbon contaminants 14 in the vadose zone 38 where the potential for such fire and explosion hazards exists. The subsurface vapor-inerting system 200 includes an inert gas assembly 202 for dispensing of an inert gas 22; and a well component assembly 220 for transferring the inert gas 22 to the contaminated geologic formation 30. The well component assembly 220 of the vapor-inerting system 200, as shown in Figures 5 and 6 , is installed close to the well component assembly 150, and is constructed in a similar manner to well component assembly 150, except for the screened PVC section 226 interval within the well riser 222 which is no more than 1ft to 10ft above the seasonal high of water table level 28. The compressed inert gas 22 such as argon gas (A) is dispensed within the well 222 in a manner so as to maintain the gravitational flow or passive flow of the argon gas 22 into the vadose zone 38 of the geologic media 30 in order to reduce any potential for fire and explosions in the vadose zone 38. This vapor-inerting system 200 is also used to provide an improved mechanism for the enhancement of anaerobic bioremediation processes, as the argon gas 22 enables the maintenance of anaerobic conditions within the contaminated geologic media being remediated.

The inert gas assembly 202 includes an inert gas cylinder 204 having a gas regulator 206 with a gas regulator valve 208, a shut-off valve 210, an outlet connection component 212 and pressure in-line gauges 214a and 214b for maintaining a precise outlet pressure to properly blanket the vadose zone 38 with the argon gas (A) 22 suppressing vapors produced by the subsurface contaminants. Gas



regulator 206 in addition includes a regulator gauge protection cage 216, and flexible tubing 98 for discharging of the argon gas (A) 22 into the well 222.

The well component assembly 220, as shown in Figures 5 and 6, includes a PVC well riser 222 having an upper end section 224 and a lower end PVC screen section 226. In addition, well component assembly 220 includes a separate subsurface housing unit 252 made of concrete or metal for containing both the inert gas assembly 202 and well component assembly 220 therein. Housing 252 includes an outer cylindrical bentonite layer 254, and an outer manhole cover 258. In addition, sub-surface housing 252 includes inner and outer surface walls 266 and 268 and a bottom wall member 270 being a gravel layer. Bottom wall member 270 includes a circular hole opening 276 within the gravel layer for receiving the upper end section 224 of well casing 222. The PVC well riser 222 is surrounded by backfilled soil cuttings 230 and a bentonite seal 232 at the upper end section 224 of the well PVC riser 222; and is surrounded by a Morie (or equivalent) sand pack 234 at the lower end section 226 of the well PVC riser 222. In addition, the well PVC riser 222 includes an inlet opening 236, a plurality of discharge side outlet openings 236s and a bottom end cap 269. Inlet opening 236 includes a well cap 238 having a disconnect coupling/fitting 242 thereon.

As shown in Figure 7, the preferred chemical nutrient composition 40 and alternative chemical nutrient compositions 41, 42, 43, 44 and 45 can be used in dual or other multiple dispensing

apparatus assemblies 80 and 80', with dual or multiple electronic assemblies 130 and 130' for the predetermined alternate feeding of the preferred composition 40 and alternative compositions 41, 42, 43, 44 and 45 into the well component assembly 150 via inlet opening 166. This allows the preferred and alternative chemical nutrient compositions 40, 41, 42, 43, 44 and 45 to be received into the contaminated geologic media 30 in precisely timed pulses in a manner which enables the temporal cycling of redox pathways so as to optimize the growth and health of MRP anaerobic microorganisms and to optimize contaminant degradation by such microorganisms 12.

#### OPERATION OF THE PRESENT INVENTION

In operation, the anaerobic bioremediation system 10 facilitates the anaerobic biodegradation, detoxification, and/or transformation of contaminant compounds such as petroleum hydrocarbons, halogenated solvents, polychlorinated biphenyls, dioxins, pesticides, cyanides, toxic metals, hazardous wastes and the like that have been released into surface environments and/or subsurface geologic media 30 whereby such contaminants are transformed into safe, less-toxic and/or harmless end-products.

In addition, the bioremediation system 10, also facilitates the anaerobic biodegradation, detoxification, and/or transformation of toxic organic and inorganic compounds in contaminated geologic media 30 under a wide range of reducing redox conditions and anaerobic respiration pathways including denitrification, manganese-reduction, iron-reduction and sulfate-reduction. Another application of the use of the anaerobic bioremediation

system 10, with regard to the above, would be to facilitate the suppression of hydrogen sulfide ( $H_2S$ ), related sulfides, mercaptans and the undesirable odors related to these compounds produced as a result of the metabolic activity of sulfur-reducing microorganisms via the stimulation and maintenance of denitrifying, manganese-reducing and iron-reducing conditions.

The well component assembly 150 provides a proper fluid-exchange interface with the contaminated geologic media 30 which in turn provides a means of infiltration of the chemical nutrient composition 40, optional water feed 20, and carrier gas 22 within the contaminated geologic formation 30 for stimulating and facilitating the bioremediation of the toxic organic and inorganic compounds by the indigenous anaerobic microorganisms 12 located within the aforementioned geologic formation 30.

In addition, the present invention also provides for an improved subsurface vapor-inerting system 200 designed to reduce oxygen gas ( $O_2$ ) concentrations 24 in the vadose-zone 38 of the contaminated geologic media 30 in order to reduce or eliminate the potential for flash fires and/or explosion hazards in the subsurface areas where the potential for such fire and explosion hazards exists. The well component assembly 220 of the vapor-inerting system 200, as shown in Figure 5, is installed adjacent to the well component assembly 150. The PVC well riser 222 of well component assembly 220 delivers the inerting argon gas 22 to the vadose zone 38 of the contaminated geologic media 30 while simultaneously the PVC well riser 152 of well component assembly

150 delivers the chemical nutrient composition 40 and the argon carrier gas 22 to the contaminated area of geologic media 30 in order to stimulate the indigenous anaerobic microorganisms 12 at that interface area 170 within the aforementioned contaminated geologic media 30. The vapor inerting system 200 can also be used independently of the anaerobic bioremediation system 10.

#### INITIAL OPERATION OF THE SYSTEMS AFTER CONSTRUCTION

To put the bioremediation processing apparatus 50 and the vapor inerting system 200 into operation initially after construction, several preparation steps are provided for, such as the construction and placement of well component assemblies 150 and 220 at the contaminated geologic site 30, construction and placement of each housing member 52 and 252 at a subsurface level within the soil strata of the contaminated geologic media 30, and the placement of each well component assemblies 150 and 220 within each housing member 52 and 252 via circular hole openings 76 and 276 each for receiving the upper end sections 154 and 224 of well piping 152 and 222, respectively, as shown in Figure 5 of the drawings.

The chemical nutrient composition 40 is made-up in a large make-up mixing tank (not shown) or in the actual product tank canisters 100a to 100f at a convenient off-site premise or at the contaminated geologic site 30 when logistics permit it by adding the dry chemicals, the liquid chemicals and diluent together in the proper order, and mixing them thoroughly. If the chemical nutrient composition 40 is made-up in the larger mixing tank, the nutrient

composition 40 is then transferred to the plurality of product tank canisters 100a to 100f via a portable pump (not shown). After the product tanks 100a to 100f are filled with the nutrient composition 40, their respective lids 102a to 100f are closed shut and the product tanks 100a to 100f are then pressurized by argon gas (A) 22 thereby sealing their respective lids 102a to 102f to prevent leaks of the nutrient composition 40 contained therein. The product tank canisters 100a to 100f are then transported to the contaminated geologic site 30.

Another preparation step is the development of a computer program for the logic controller 132 of the electronic control assembly 130 where the algorithms in the program are based on mathematical models of one or more of the following operating parameters for the bioremediation system 10:

- 1) The rates of the diffusion-based transport of the biogeochemical compounds in the chemical nutrient composition 40 being used in the contaminated geologic media 30.
- 2) The uptake rates of biogeochemical compounds in the chemical nutrient composition 40 by the naturally occurring MRP microorganisms 12 in-situ for degrading the contaminants.
- 3) The rates, duration and repetition of cycles for
  - a) biomass growth followed by
  - b) biomass decay in-situ.
- 4) The duration and repetition and/or cycling of various

## redox pathways

In conjunction with the preceding step, a plurality of digital and/or fiber-optic sensors 144a to 144i may be connected to logic controller 132 and regularly calibrated to monitor and/or control the delivery of the chemical nutrient composition 40 based upon real-time measurements of one or more parameters in-situ at selected locations within the contaminated geologic media 30. For example, such parameters may include in-situ monitoring of the following:

- 1) Static-water levels;
- 2) Meteorologically induced changes in static-water levels;
- 3) The rates each of the electron-acceptors are being used by the MRP microorganisms 12;
- 4) The biomass growth rate of the naturally occurring MRP microorganisms 12 in-situ;
- 5) The relative metabolic activity of the naturally occurring MRP microorganisms 12 in-situ;
- 6) The conversion rates of the converted end-products being generated by the MRP microorganisms 12;
- 7) The pH and/or redox potential within the contaminated geologic media 30;
- 8) The temperature within the contaminated geologic media 30.

The operator (being at the site) removes the outer manhole cover 58 having a foam insulation 60 attached from the top of the subsurface apparatus housing 52 allowing the operator access to the

well component assembly 150 within housing 52. The operator then places the plurality of product tanks 100a to 100f, and the electronic control assembly 130 within the subsurface housing 52, such that the product tanks 100a to 100f are placed on the bottom floor 72 in a circular fashion such that the last product tank 100f having the electronic control assembly 130 attached thereto is adjacent and in close proximity to the well component assembly 150. The product tanks 100a to 100f are then joined together via the plurality of quick disconnect couplings 104a to 104f having the jumper-line tubing 106a to 106f attached thereto, if being operated in series as shown in Figure 3a. If being operated in parallel as shown in Figure 3b, the jumper line tubing is connected from the manifolds 99a and 99b to the product tanks 100a to 100f. Once all of the digital and/or fiber optic sensors 144a to 144i have been calibrated (if the optional sensors are to be used) and the logic controller 132 is properly programmed, the operator then connects the battery power pack 142 via electrical power line 140 for supplying electrical power to the logic controller 132. The operator then connects the logic controller 132 to the last product tank 100f via quick disconnect coupling 104f (for series operation). The gas cylinder 82 having argon gas (A) 22 (which is still located at ground level 26 above the apparatus system 50 for easier access) is then connected to the first product tank 100a (for series operation) or to the gas manifold 99a (for parallel operation) via the quick disconnect coupling 104a. The operator now opens the valve 88 on gas cylinder 82 and the gas pressure, as

shown on the in-line gas pressure gauge 92, is set to be within the range of 5-to-10 psig or approximately 5 psig above the minimum pressure required via gas regulator valve 86 on the gas regulator assembly 84 to initiate the flow of the chemical nutrient composition 40 out of the product tanks 100a to 100f with the argon carrier gas (A) 22 and into the product tubing 148. Simultaneously, as the above step is taking place, the operator also checks that the logic controller 132 is manually actuated and the ball valve 114 is opened, and the logic controller 132 is set and adjusted to the desired flow of chemical nutrient composition 40 out of the controller 132 and into the product tubing 148. Once the nutrient composition 40 is flowing freely through the dispensing apparatus assembly 80 of the bioremediation processing apparatus 50 and out the end of product tubing 148, the logic controller program component 134 and timing element 136 of logic controller 132 are checked again or modified if desired by the operator. Then the logic controller 132 is manually closed and set to an automatic setting by the operator. The product tubing 148 is then placed into the well apparatus 150. The gas cylinder 82 which is already connected to the first product tank 100a or gas manifold 99a is then placed into subsurface housing 52 next to the first product tank 100a or gas manifold 99a.

As noted above, if the product tanks are to be operated in parallel as shown in Figure 3B, the gas cylinder 82 is connected to the gas manifold 99a, and jumper tubing from the inlet of each product tank is connected to the gas manifold. Jumper lines are



then connected to the discharge of each product tank at one end and to the liquid manifold 99b on the other end, as shown in Figure 3B. The rest of the set-up process as described above for series operation, is the same for parallel product tank operation.

5        If the optional water feed is installed and is to be used, manual control valve 114' would be opened and adjusted until the desired flow rate of water is discharged from tubing 115. The automatic control valve 138' contained within water feed logic controller 132' would then be manually closed, and the logic controller programmed to operate as needed. Water feed tubing 115 would then be placed into the well apparatus 150.

10        The man hole cover 58 having the insulation layer 60 is then placed back on top of the subsurface housing 52 and the anaerobic bioremediation system 10 is now operating to facilitate the biodegradation, biotransformation or detoxification of the contaminants 14 at interface area 170 of contaminated media 30 into harmless end products including carbon dioxide gas ( $\text{CO}_2$ ) 16, nitrogen gas ( $\text{N}_2$ ) 18 and water ( $\text{H}_2\text{O}$ ) 20.

20        In the event that an above-grade installation for the bioremediation system 10 is preferred, the installation and set-up procedures for the dispensing apparatus 80 would be the same as described above with the exception that the said dispensing assembly would be installed in a suitable above-ground housing.

25        If the vapor suppression and inerting system 200 is being used in conjunction with the dispensing apparatus assembly 80, the operator removes outer manhole cover 258 and places the inert gas

assembly 202 in close proximity to the well component assembly 220,  
such that the inert gas cylinder 204 having argon gas (A) 22  
therein stands on the bottom gravel floor 272 of housing 252. The  
operator then checks the gas regulator 206 and the component  
fitting 212 and attaches tubing 98 to well cap disconnect fitting  
242 on well cap 238, so that the flexible discharge tubing 98 can  
discharge the argon gas (A) 22 into the well riser 222 of well  
component assembly 220. The operator then opens valve 210 on gas  
cylinder 204 and adjusts the gas regulator valve 208, such that the  
argon gas (A) 22 is set to flow and run at the minimum setting at  
which the argon gas (A) 22 is discharged into the well riser 222.  
The minimum measurable rate on the pressure gauge 214 of gas  
regulator 206 for discharging the argon gas (A) 22 into the well  
riser 222 has a setting of about 1 psig.

The purpose of gas cylinder 204 is to maintain the  
gravitational flow or passive flow of the argon gas (A) 22 into the  
vadose zone 38 of the contaminated geologic media 30 in order to  
suppress vapors from the subsurface contaminants which have the  
potential to cause fire and explosions in that area. Also, the  
argon gas (A) 22 enables the maintenance of anaerobic conditions  
within the contaminated geologic site 30 for a continuous  
enhancement of the anaerobic bioremediation processes taking place  
at interface area 170. The manhole cover 258 is then placed on  
housing 252 by operator for the completion of this step of  
initially activating the vapor suppression system 200. In the  
event that an above-grade installation for the vapor suppression



130 for proper functioning according to the pre-determined standards initiated for the anaerobic bioremediation system 10 in degrading the contaminants 14 at the geologic work site 30. These checks are generally performed at the same time that the product tanks 100a to 100f and gas cylinders 82 and 204 are replaced.

When the operator decides it is time to replace the product tanks 100a' to 100f' based on preset feed rates and frequency, the manhole cover 58 is removed from the subsurface housing 52 allowing access to the dispensing apparatus assembly 80 and the electronic control assembly 130 within subsurface housing 52, as shown in Figures 1 and 5 of the drawings. The operator then closes the shut-off valve 88 on gas cylinder 82 and removes the quick disconnect coupling 104a connecting gas cylinder 82 having argon gas (A) 22 from the first product tank 100a in-line. Then the gas cylinder 82 is lifted out of the housing 52.

The operator then removes the quick disconnect coupling 104l connecting the last product tank 100f to the tubing 106f from the logic controller 132, such that the logic controller 132 is then disconnected and set aside. If the logic controller 132 is in an open mode, the operator then manually closes the automated control ball valve 138. The operator then continues to remove and set aside the remaining quick disconnect couplings 104b to 104k and jumper line tubing 106a to 106f from the empty product tanks 100a to 100f that were connected in series, as shown in Figures 2 and 3 of the drawings. The empty product tanks 100a to 100f are then removed from the subsurface housing 52 and are set aside at ground level 26.

The replacement product tanks 100a' to 100f' having nutrient composition 40 therein are then placed onto the bottom floor 72 of the subsurface housing 52 in a circular fashion, as shown in Figure 2 of the drawings. The operator then puts back on all of the quick disconnect couplings 104b to 104k and jumper line tubing 106a to 106f onto the replacement product tanks 100a' to 100f' in order to connect these product tanks 100a' to 100f' in series. Then the operator puts back on the quick disconnect coupling 104l onto the last product tank 100f', where coupling 104l connects to barb connector 110 for reattaching the logic controller 132 to the last product tank 100f'. Next, the operator sets a replacement carrier gas cylinder 82' of compressed argon gas (A) 22 at ground level 26 and re-attaches the quick disconnect coupling 104a and the hose clamp 112 on hose 98 thereby connecting the gas cylinder 82 to the first product tank (in-line) 100a'. The operator now re-opens the valve 88 and adjusts the regulator valve 86 on the gas regulator assembly 84, such that the in-line pressure gauge 92 of gas regulator 84 is set to a 5 psig setting, or set at approximately 5 psig above the minimum pressure required to initiate the flow of the chemical nutrient composition 40 out of the product tanks 100a' to 100f'. The operator now adjusts the logic controller 132 such that automatic ball valve 138 is manually re-set to the opened position, and where the manual ball valve 114 attached on the discharge side of the logic controller 132 is re-set to adjust the nutrient composition 40 flow at a desired rate for flowing into the geologic media 30. Once the nutrient composition 40 is flowing freely

through the dispensing apparatus assembly 80 and out of product tubing 148, the operator then manually closes automatic valve 138 and re-checks the logic controller program component 134 for the feed algorithm previously inputted. The logic controller 132 is then manually closed and set to an automatic setting by the operator. The operator then places product tubing 148 into well assembly 150 and places gas cylinder 82 (which is already connected to the first product tank 100a), into subsurface housing 52. Lastly, the operator again replaces the manhole cover 58 having insulation 60 on top of the subsurface housing 52 to prevent freezing of the nutrient composition 40 during the winter months.

If the vapor suppression system 200 is in use, manhole cover 258 is removed and the shut off valve 210 on gas cylinder 204 is closed. Tubing 98 is then disconnected from well cap 238 via quick disconnect coupling 242, and then the gas cylinder 204 is lifted out of the housing 252 by the operator.

The replacement inert gas cylinder 204' is then placed by the operator back onto the bottom gravel floor 272 of the subsurface housing 252 and adjacent to well component assembly 220, as shown in Figure 5 of the drawing. The operator then checks the gas regulator 206, the connection fitting 212 via tubing 98 being properly attached to the inert gas cylinder 204 and attaches tubing 98 to the well cap quick disconnect fitting 242 on well cap 238, as shown in Figure 5 of the drawings. The operator then adjusts the valve 210 on gas cylinder 204 to an open position, and adjusts the valve 208 on gas regulator assembly 206 such that the argon gas (A)

22 is set to flow and run at the minimum setting at which the argon gas (A) 22 is discharged into the well riser 222. The minimum measurable rate on the pressure gauge 216 of gas regulator 206 for discharging the argon gas (A) 22 into the well riser 222 has a setting of about 1 psig.

The continuous operation of the bioremediation processing apparatus 50 of the anaerobic bioremediation system 10 for degrading contaminants 14 at a particular geologic site 30 may take up to several months. During this time, operators of the anaerobic bioremediation system 10 will repeat the aforementioned steps and procedures of replacing the gas cylinders 82 and 204 having argon gas (A) 22 therein and product tanks 100a to 100f having chemical nutrient compositions 40 to 45 therein numerous times until the project has been completed. Also, the operators on an ongoing basis will periodically re-calibrate the electronic control assembly 130 for the proper flow of the nutrient compositions 40 to 45 into the interface area 170 of geologic media 30.

In the event that an above-grade installation for the anaerobic bioremediation system 10 and the vapor suppression system 200 is utilized, the installation and set-up procedures for the dispensing apparatus 80 and inert gas assembly 202 would be the same as described above with the exception that the said dispensing apparatus and inerting assembly would be operated and maintained within a suitable above-ground housing.

## HYDROGEN SULFIDE GENERATION AND SUPPRESSION

As noted in the Overview of the invention, bacterial utilize electron acceptors in the order of their decreasing energy yield (Gibbs Free Energy [ $\Delta G$ ] in KJ/mole  $\text{CH}_2\text{O}$ ). In theory, as the availability of a higher-energy electron acceptor wanes (e.g.,  $\text{O}_2$ ), conditions become favorable for microbial respiration with lower-energy electron acceptors (e.g.,  $\text{NO}_3$ ,  $\text{MN(IV)}$ ,  $\text{Fe(III)}$ , and  $\text{SO}_4$ ). The natural sequence of microbial utilization of electron acceptors in the environment is summarized in Table 1. In the absence of higher-energy electron acceptors, sulfates serve as the electron acceptor for oxidation by anaerobic microorganisms. Sulfate is reduced to hydrogen sulfide ( $\text{H}_2\text{S}$ ) under anaerobic conditions; hydrogen sulfide has a characteristic rotten egg odor, is extremely toxic, and is corrosive to metals. Hydrogen sulfide can also be biologically oxidized to sulfuric acid which is also extremely corrosive.

By providing a preferential electron acceptor (such as oxygen in the form of nitrous oxide, nitrate,  $\text{Fe(III)}$  or  $\text{Mn (IV)}$ ) where there is potential for  $\text{H}_2\text{S}$  formation due to the presence of sulfate, bacteria will preferentially use the higher-energy yielding electron acceptor, and  $\text{H}_2\text{S}$  will not be formed. The result is suppression of malodorous and problematic compounds that would otherwise be produced.



EXAMPLES OF USE FOR THE  
ANAEROBIC BIOREMEDIATION SYSTEM

EXAMPLE 1: ACTIVE SERVICE-STATION SITE  
GASOLINE RELEASE, NORTHERN NEW JERSEY

**Site Description and Type of Release**

A release of an unknown quantity of gasoline occurred from piping associated with the former UST systems removed and replaced at the site in the late 1980's.

**Site Hydrogeology**

The overburden sediments at the site comprise fine-to-medium glaciofluvial sands with varying amounts of silt. The water table ranges from 6-to-10 ft. below grade. A compact, silty-clay aquitard of glaciolacustrine origin is present at depths ranging from 15-to-19 ft. below grade. Bedrock was not encountered during drilling and the bedrock aquifer is not believed to have been impacted. A stream wraps around the site and its surroundings approximately 200 ft. downgradient and ground-water flow conditions have been interpreted fluctuate in response to local surface-water/ground-water interaction and meteorological events.

**Investigation and Remediation**

A subsurface investigation was performed by Geovation from 1993-1996 which included the installation of seven monitor wells and collection of soil and ground water samples. The results of the investigation indicated the presence of gross soil and ground-water contamination, with as much as 2.1 ft. of gasoline in a site monitor well. Two anaerobic bioremediation systems (ABSs) were installed, one next to the pump island and one adjacent to the tank field, with

minimal disruption of site commercial activities. Operation of the ABSs has had a negligible impact on gas-station operations.

Baseline contaminant levels included more than 2 ft. of free product, MTBE- and 1,2-DCA-amended gasoline in the most contaminated well and >16-42 mg/L total BTEX in the next most contaminated well.

#### Results

To date, free product thicknesses have decreased approximately 90 percent and dissolved phase BTEX compounds have decreased by as much as 88 percent in the site well with the highest BTEX level prior to remediation activities. BTEX concentrations in this well decreased by 92% over the first three months of treatment from >16 mg/L to 3.4 mg/L. Microbiological assays and microscopic investigation documented a rapid response of the microbial community to ABS treatment as evidenced by a 30-fold increase in bacterial biomass in the downgradient monitor well closest to one of the ABSs, by the second week of treatment. The analysis of biochemical markers, including phospholipid fatty acid (PLFA) analyses, indicated that ABS treatment had increased the biomass and improved the overall health and growth rate of the microbial community. These data also indicated a shift in the composition of the microbial community towards gram-negative, more rapidly growing species in response to ABS treatment. Gene probe analysis of the microbial community indicated the presence of strict anaerobes, such as *Geobacter* spp. and the like. Additional assays are underway to further investigate the anaerobic microbial community at the site.

EXAMPLE 2: FUEL OIL RELEASE AT  
A FORMER BANK, BROOKLYN, NEW YORK

**Site Description and Type of Release**

5           Two separate releases of fuel oil occurred totalling on the order of 3,500 gal. from a UST used to heat the facility.

**Site Hydrogeology**

10           Overburden sediments at the site are predominantly medium to coarse sands associated with glacial-outwash and/or a kame-delta, with ground water at approximately 17 ft. below grade. Bedrock was not encountered and it is not believed to have been impacted.

**Investigation and Remediation**

20           Upon discovery of the release, the property owner's consultant removed contaminated soils from the source area and installed a series of monitor/recirculation wells for a carbon treatment system. Several pump-and-treat system wells were installed in the basement of the building and significant amounts of free product were detected and recovered. Monitoring data indicated that most of the remaining free-product contamination was trapped beneath the building. Extensive pump and treat efforts were met with limited effectiveness in that free-product recovery waned and free-product persisted in monitor wells across the site. Accordingly, the engineering firm in charge evaluated several bioremediation alternatives and Geovation was selected to conduct an anaerobic bioremediation program to augment the ongoing remediation activities. Three of Geovation's anaerobic bioremediation systems (ABS) 10 were installed at key locations in the contaminant plume

and operated beginning in late November 1996.

## Results

Prior to remediation in November 1996, free-product thicknesses in site monitor wells were on the order of 0.35 ft. From late November through early December, several storm events occurred which caused product levels in site wells to increase to approximately 0.5 ft. in thickness as the water table rose through contaminated vadose-zone soils. As of late February 1997, approximately 3 months after initiation of remediation activities and approximately 2 weeks after cessation of the initial anaerobic bioremediation treatment program, free product was eliminated in most site wells, with only a sheen observed in several downgradient locations. Additional ABS operation initiated in late March 1997 has eliminated residual free-product in site wells.

Throughout the course of the ABS operation, several obvious indications of enhanced biological activity were documented including the "carbonation" of site ground water resulting from carbon dioxide produced by the microbial community as well as documented increases in the number and size of bacteria present in site monitor wells. A twenty-to-thirty-fold increase in biomass was documented over the first two weeks of ABS operation. The most dramatic increases in microbial biomass occurred in the wells with the highest levels of contamination. The presence of a biomass slurry was routinely observed in site monitor wells and in the oil/water separator tanks associated with the pump and treat system. ABS operation is continuing at this time to address remnant

dissolved phase contamination.

#### ADVANTAGES OF THE PRESENT INVENTION

An advantage of the present invention is that it provides for an anaerobic bioremediation system for the anaerobic biodegradation, detoxification and transformation of toxic organic and inorganic contaminants within contaminated geologic media into non-toxic compounds without further production of regulated wastes or other undesirable by-products that effect air, water and soil at the geologic site.

Another advantage of the present invention is that it provides for an anaerobic bioremediation system for in-situ treatment of geologic media containing organic and inorganic contaminants that are metabolizable or transformable by naturally occurring indigenous, MRP anaerobic bacteria including denitrifying-, manganese-, iron- and sulfate-reducing microorganisms within the contaminated geologic media at the site.

Another advantage of the present invention is that it provides for an anaerobic bioremediation system having apparatus which enables the delivery of electron acceptors, nutrients, chelating agents, surfactants, a diluent and an inert carrier gas to promote the growth of indigenous, MRP anaerobic bacteria including denitrifying-, manganese-, iron- and sulfate-reducing microorganisms such that the metabolism or transformation of the contaminants by these microorganisms can easily take place without the use of implanted microorganisms at the contaminated site.

Another advantage of the present invention is that it provides for an anaerobic bioremediation system having apparatus which enables the electron acceptors, nutrients, chelating agents, surfactants, a diluent and an inert carrier gas to be more readily and rapidly dispersed in the contaminated geologic media, thereby becoming more widely available to MRP anaerobic bacteria within the contaminated geologic media.

Another advantage of the present invention is that it provides for an anaerobic bioremediation system the electron acceptors, nutrients, chelating agents, surfactants, a diluent and an inert carrier gas in a chemical composition and form that is readily utilizable by indigenous, MRP anaerobic bacteria including denitrifying-, manganese-, iron- and sulfate-reducing microorganisms within the contaminated geologic media.

Another advantage of the present invention is that it provides for an anaerobic bioremediation system that has the means to supply electron acceptors, nutrients, chelating agents, surfactants, a diluent and inert carrier gas as well as the capacity to modify the pH, redox potential and the availability of oxygen in the subsurface geologic media whereby the use of sodium hexametaphosphate and/or similar ringed and/or linear polyphosphates as the exclusive source of phosphate and the use of nitrate as both an electron acceptor and the main source of assimilated (i.e., nutrient) nitrogen, taken together with other unique aspects of the chemical compositions, enables a major improvement for the stimulation and growth of indigenous MRP microorganisms within contaminated geologic media.

Another advantage of the present invention is that it provides a means for stimulating several different microbial respiration pathways under different redox conditions in-situ by using one or more chemical compositions in such a manner so as to stimulate MRP anaerobic bacteria including denitrifying-, manganese-, iron- and sulfate-reducing microorganisms within the contaminated geologic media.

Another advantage of the present invention is that it provides for an anaerobic bioremediation system having a means for stimulating, alternating and/or cycling various redox conditions and microbial respiration pathways within the contaminated geologic media in-situ by using one or more chemical compositions in such a manner so as to stimulate a temporal and/or spatial succession of redox conditions in the subsurface in order to enhance the growth of MRP anaerobic microorganisms and to optimize contaminant biodegradation and/or biotransformation by such microorganisms.

Another advantage of the present invention is that it provides for an anaerobic bioremediation system having the capability for reducing flash fire and/or explosion hazards in the contaminated geologic media.

Another advantage of the present invention is that it provides for an anaerobic bioremediation system having apparatus that is simple and inexpensive to construct and use, and which enables efficient delivery and monitoring of the nutrients and electron acceptors for the optimum growth rate and kinetics of various indigenous, denitrifying and other MRP microorganisms in order to

maximize the rate of degradation and transformation of the contaminants into non-toxic compounds by these indigenous MRP microorganisms.

Another advantage of the present invention is that it provides for an anaerobic bioremediation system having a process that is simple and inexpensive to operate, especially in the field on a large scale basis.

Another advantage of the present invention is that it provides for an anaerobic bioremediation system whose operation can be modified manually or automatically either on-site or from off-site locations based on real-time measurements of in-situ conditions.

Another advantage of the present invention is that it provides for an anaerobic bioremediation system having a process that can be performed rapidly and safely in the field and which results in the site meeting environmental clean-up standards set by various governmental agencies more rapidly and at a lower cost than can be accomplished with other methods.

A further advantage of the present invention is that it provides for an improved anaerobic bioremediation system that can be easily produced in an automated and economical manner and is readily affordable by various responsible parties, consultants, contractors, engineers, governmental agencies and corporate users.

A latitude of modification, change, and substitution is intended in the foregoing disclosure, and in some instances, some features of the invention will be employed without a corresponding use of other features. Accordingly, it is appropriate that the



appended claims be construed broadly and in a manner consistent with the spirit and scope of the invention herein.

WHAT IS CLAIMED IS:

1. A liquid chemical composition for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in contaminated geologic media, comprising:

5 a) sodium nitrate in the range of one-fifth (0.2) to four (4) pounds per gallon of said chemical composition;

b) sodium hexametaphosphate or other biologically hydrolyzable ring or linear polyphosphate in the range of one twentieth (0.05) to five (5) pounds per gallon of said chemical composition;

10 c) a surfactant in the range of 0.01% to 10% by volume of said chemical composition; and

d) a diluent.

15 2. A chemical composition in accordance with Claim 1, further including one or more of the following chelating agents selected from the group consisting of citric acid, sodium citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA) and equivalents thereof; in the range of one twentieth (0.05) to  
20 three (3) pounds per gallon of said chemical composition.

3. A chemical composition in accordance with Claim 1, wherein the molar ratios of nitrate ( $\text{NO}_3^-$ ) to phosphorus (P) are in the range of 2:1 to 50:1.

4. A chemical composition in accordance with Claim 1, further including an inert gas as a carrier for said chemical composition.

5. A chemical composition in accordance with Claim 4, wherein said inert gas is argon, neon, krypton or xenon.

6. A liquid chemical composition for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in contaminated geologic media, comprising:

- a) sodium nitrate in the range of zero (0) to four (4) pounds per gallon of said chemical composition;
- b) nitrous oxide in the range of one one hundredth (0.01) to one fiftieth (0.02) pounds per gallon of said chemical composition;
- c) sodium hexametaphosphate or other biologically hydrolyzable ring or linear polyphosphate in the range of one two hundredth (0.005) to five (5) pounds per gallon of said chemical composition;
- d) a surfactant in the range of 0.01% to 10% by volume of said chemical composition; and
- e) a diluent.

7. A chemical composition in accordance with Claim 6, further including one or more of the following chelating agents selected from the group consisting of citric acid, sodium citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA)

and equivalents thereof; in the range of one twentieth (0.05) to three (3) pounds per gallon of said chemical composition.

8. A chemical composition in accordance with Claim 6, wherein the molar ratios of the combined nitrous oxide and nitrate nitrogen (N) to phosphorus (P) are in the range of 0.5:1 to 50:1.

9. A chemical composition in accordance with Claim 6, further including an inert gas as a carrier for said chemical composition.

10. A chemical composition in accordance with Claim 9, wherein said inert gas is argon, neon, krypton or xenon.

11. A chemical composition in accordance with Claim 1, further including nitrous oxide ( $N_2O$ ) gas as a supplemental chemical for direct infiltration into the contaminated media.

12. A liquid chemical composition for anaerobic biodegradation, detoxification and transformation of toxic organic and inorganic compounds in geologic media, comprising:

a) a sulfate-containing ( $SO_4^{2-}$ ) electron acceptor chemical compound in the form of ferrous sulfate heptahydrate, sodium sulfate or other soluble salts of sulfate in the range of one-half (0.5) to four (4.0) pounds per gallon of said chemical composition;

b) sodium hexametaphosphate or other biologically

hydrolyzable ring or linear polyphosphate in the range of one twentieth (0.05) to five (5) pounds per gallon of said chemical composition;

c) a surfactant in the range of 0.01% to 10% by volume of said chemical composition; and

d) a diluent.

13. A chemical composition in accordance with Claim 12, further including one or more of the following chelating agents selected from the group consisting of citric acid, sodium citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA) and equivalents thereof; in the range of one twentieth (0.05) to three (3) pounds per gallon of said chemical composition.

14. A chemical composition in accordance with Claim 13, wherein the molar ratios of sulfate ( $\text{SO}_4^{2-}$ ) to phosphorus (P) are in the range of 2:1 to 50:1.

15. A chemical composition in accordance with Claim 12, further including an inert gas as a carrier for said chemical composition.

16. A chemical composition in accordance with Claim 15, wherein said inert gas is argon, neon, krypton or xenon.

17. A liquid chemical composition for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in geologic media comprising:

a) a primary compound acting as a source of both nutrient nitrogen and two forms of electron acceptor in the form of ferric nitrate nonahydrate in the range of one-half (0.5) to four (4.0) pounds per gallon of said chemical composition.

b) a biologically usable phosphate in the form of sodium hexametaphosphate or other biologically hydrolyzable ring or linear polyphosphate in the range of one twentieth (0.05) to five (5) pounds per gallon of said chemical composition; and

c) a surfactant in the range of 0.01% to 10% by volume of said chemical composition; and

d) one or more of the following chelating agents selected from the group consisting of citric acid, sodium citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA) and equivalents thereof in the range of one twentieth (0.05) to three (3) pounds per gallon of said chemical composition; and

e) a diluent.

18. A chemical composition in accordance with Claim 17, wherein the molar ratios of the combined nitrate and ferric iron  $[\text{NO}_3^-$  and  $\text{Fe(III)}]$  to phosphorus (P) are in the range of 2:1 to 50:1.

19. A chemical composition in accordance with Claim 17, further including an inert gas as a carrier for said chemical composition.

5 20. A chemical composition in accordance with Claim 19, wherein said inert gas is argon, neon, krypton or xenon.

10 21. A liquid chemical composition for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in geologic media, comprising:

15 a) a primary compound acting as a source of electron acceptor nitrogen, nutrient nitrogen and micronutrient manganese, in the form of manganese nitrate, manganese nitrate tetrahydrate, or manganese nitrate hexahydrate in the range of one-half (0.5) to four (4.0) pounds per gallon of said chemical composition;

b) a biologically usable phosphate in the form of sodium hexametaphosphate or other biologically hydrolyzable ring or linear polyphosphate in the range of one twentieth (0.05) to five (5) pounds per gallon of said chemical composition;

20 c) a surfactant in the range of 0.01% to 10% by volume of said chemical composition; and

d) a diluent.

25 22. A chemical composition in accordance with Claim 21, further including one or more of the following chelating agents selected from the group consisting of citric acid, sodium citrate,

nitritotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA) and equivalents thereof; in the range of one twentieth (0.05) to three (3) pounds per gallon of said chemical composition.

5           23. A chemical composition in accordance with Claim 21, wherein the molar ratios of combined nitrate and manganese to phosphorus are in the range of 2:1 to 50:1.

10           24. A chemical composition in accordance with Claim 21 further including an inert gas as a carrier for said chemical composition.

          25. A chemical composition in accordance with Claim 24, wherein said inert gas is argon, neon, krypton or xenon.

15           26. A liquid chemical composition for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in contaminated geologic media comprising:

20           a) a chelating agent which makes transition metals, including, but not limited to Mn(IV) and Fe(III) present in the geologic media more biologically available to MRP microorganisms, with this chelating agent selected from the group which includes citric acid, sodium citrate, nitritotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA) and equivalents thereof; in the range of one twentieth (0.05) to three (3) pounds per gallon of  
25           said chemical composition.

          b) a biologically usable phosphate in the form of sodium



hexametaphosphate or other biologically hydrolyzable ring or linear polyphosphate in the range of one twentieth (0.05) to five (5) pounds per gallon of said chemical composition;

c) a surfactant in the range of 0.01% to 10% by volume of said chemical composition; and

d) a diluent.

27. A chemical composition in accordance with Claim 26, further including an inert gas as a carrier for said chemical composition.

28. A chemical composition in accordance with Claim 27, wherein said inert gas is argon, neon, krypton or xenon.

29. Bioremediation apparatus for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in a contaminated geologic media, comprising:

a) a first set of one or more storage tanks containing a chemical composition for anaerobic biodegradation of toxic compounds in contaminated geologic media;

b) valve means connected to said first set of storage tanks;

c) at least one logic controller means for opening and closing said valve means connected to said first set of storage tanks to supply said chemical composition to the contaminated geologic media; and

d) a screened well connected to said first set of storage tanks for supplying said chemical composition to the contaminated geologic media.

5           30. Bioremediation apparatus in accordance with Claim 29, wherein said one or more storage tanks contain an inert gas as a carrier for said chemical compositions.

10           31. Bioremediation apparatus in accordance with Claim 30, wherein said one or more storage tanks are pressurized for the pressurized storage and dispensing of said chemical composition and said inert gas.

15           32. Bioremediation apparatus in accordance with Claim 29, wherein said logic controller means includes means for inputting and executing an algorithm or computer program into said logic controller means.

20           33. Bioremediation apparatus in accordance with Claim 29, further including one or more sensors connected to said logic controller means.

25           34. Bioremediation apparatus in accordance with Claim 33, wherein said sensors are disposed in the contaminated geologic media for taking readings of conditions therein.

35. Bioremediation apparatus in accordance with Claim 34,  
wherein said sensors include means for sensing data to obtain  
measurements of static-water levels, the changes in static-water  
levels, the in-situ concentrations of each of the components of the  
said chemical compositions or the by products thereof, the rate of  
use of one or more of the components of the said chemical  
compositions by anaerobic microorganisms, the total estimated mass  
of the microorganisms in-situ, the biomass growth rate of the  
naturally occurring anaerobic microorganisms in-situ, the relative  
metabolic activity of the naturally occurring anaerobic  
microorganisms in-situ, the conversion rates of the converted end-  
products being generated by the anaerobic microorganisms, the pH  
and/or redox potential of the saturated geologic media or biomass,  
and the temperature of the saturated geologic media or biomass.

36. Bioremediation apparatus in accordance with Claim 33,  
further including means for transmitting the data received by said  
sensors to a data-logger or computer at a remote location.

37. Bioremediation apparatus in accordance with Claim 35,  
wherein said logic controller means further includes programming  
means for controlling the dispensing of said chemical compositions  
at predetermined dispensing rates or as a function of one or more  
of said measurements.

38. Bioremediation apparatus in accordance with Claim 37 further including means for remotely inputting an algorithm or computer program into the said logic controller means by the user for purposes of controlling the dispensing of the said chemical compositions.

39. Bioremediation apparatus in accordance with Claim 37, wherein said programming means includes a programmer component having a timing element for electronically opening and closing said valve means for the precise metering of said chemical composition into the contaminated geologic media.

40. Bioremediation apparatus in accordance with Claim 29, wherein said valve means includes an automatic ball valve being electronically connected to said logic controller means and a manual ball valve being mechanically connected to said logic controller means.

41. Bioremediation apparatus in accordance with Claim 29, further including:

- a) a pressurized water supply line;
- b) a pressure reducing valve connected to said pressurized water supply line;
- c) an alternate automatic valve means connected to said pressure reducing valve;
- d) an alternate logic controller means being

electronically connected to said alternate automatic valve means for opening and closing said alternate automatic valve means connected to said pressurized water supply line to supply said water supply to the contaminated geologic media;

5 e) an alternate manual valve means wherein said valve means is mechanically connected to said alternate logic controller means;

f) said screened well being connected to said pressurized water supply line for supplying said water supply to the  
10 contaminated geologic media.

42. Bioremediation apparatus in accordance with Claim 29, further including:

15 a) two or more sets of one or more storage tanks containing additional chemical compositions for anaerobic biodegradation of toxic compounds in the contaminated geologic media;

b) two or more valve means connected to said two or more sets of storage tanks;

20 c) one or more alternate logic controller means for opening and closing said additional valve means connected to said additional sets of storage tanks to supply said additional chemical compositions to the contaminated geologic media; and

d) said screened well being connected to said additional  
25 sets of storage tanks for supplying said alternate chemical compositions to the contaminated geologic media.

43. Bioremediation apparatus in accordance with Claim 42, wherein said logic controller means and said alternate logic controller means each include means for controlling the delivery of said chemical composition and said additional chemical compositions to the contaminated geologic media simultaneously or in an alternating manner.

44. Bioremediation apparatus in accordance with Claim 29, further including a vapor suppression system for reducing flash fire and/or explosion hazards in the contaminated geologic media, comprising:

a) one or more gas cylinder tanks containing an inert gas for reducing oxygen gas ( $O_2$ ) concentrations within the contaminated geologic media;

b) control valve means connected to said one or more gas cylinder tanks;

c) pressure sensing means connected to said one or more gas cylinder tanks for providing a minimum pressure setting in which said inert gas is discharged from said gas cylinder tanks to the contaminated geologic media; and

d) means for connecting a second well to said gas cylinder tanks for supplying said inert gas to the contaminated geologic media.

45. Bioremediation apparatus in accordance with Claim 44, wherein said inert gas is argon, neon, krypton or xenon.

46. Bioremediation apparatus in accordance with Claim 44,  
wherein said control valve means is a manual shut-off valve.

47. Bioremediation apparatus in accordance with Claim 44,  
5 wherein said pressure sensing means include one or more in-line  
pressure gauges.

48. A solid chemical composition for anaerobic  
biodegradation, detoxification, and transformation of toxic organic  
10 and inorganic compounds in contaminated geologic media, including  
one or more of the following granular Manganese (IV)-containing  
compositions, selected from the group consisting of glauconite,  
manganese greensand and pyrolusite.

49. A solid chemical composition in accordance with Claim 48  
15 wherein the Manganese (IV) contained in said granular composition  
acts as an electron acceptor for manganese-reducing bacteria and/or  
other MRP anaerobic bacteria.

50. A solid chemical composition in accordance with Claim 49  
20 wherein the composition is used as a component in a remediation  
trench, slurry-wall, semi-permeable barrier, as a well-packing  
material in a screened well, or other subsurface remedial  
application.

25

51. A solid chemical composition for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in contaminated geologic media, comprised in whole or in part with minerals containing Fe (III) including Fe(III)OH and equivalents thereof.

52. A solid chemical composition in accordance with Claim 51 wherein the Fe (III) contained in said granular composition acts as an electron acceptor for iron-reducing bacteria and/or other MRP anaerobic bacteria.

53. A solid chemical composition in accordance with Claim 52 wherein the composition is used as a component in a remediation trench, slurry-wall, semi-permeable barrier, as a well-packing material in a screened well, or other subsurface remedial application.

54. A method for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in contaminated geologic media comprising the steps of:

- a) pressurizing one or more storage tanks containing a chemical composition and an inert carrier gas;
- b) connecting valve means to said one or more pressurized storage tanks;
- c) connecting a well to said valve means for supplying said chemical composition and said inert carrier gas through said



well to the contaminated geologic media; and

d) opening and closing said valve means to dispense said chemical composition and said inert carrier gas under pressure through said well to the contaminated geologic media.

5

55. A method in accordance with Claim 54, further including the step of disposing sensors in the contaminated geologic media for taking readings of conditions therein.

10

56. A method in accordance with Claim 55, wherein the step of opening and closing said valve means is performed by logic controller means having means for inputting and executing an algorithm or computer program into said logic controller means.

15

57. A method in accordance with Claim 54, wherein the step of opening and closing said valve means is performed by logic controller means having means for inputting and executing an algorithm or computer program into said logic controller means.

20

58. A method in accordance with Claim 57, wherein the program of said logic controller means is modified via an off-site computer means.

25

59. A method in accordance with Claim 56, wherein the output from one or more said sensors directly modifies or controls the program running in said logic controller means.

60. A method in accordance with Claim 59, wherein the data from said sensors is a) transmitted to an off-site computer; and

b) analyzed and interpreted by the user; and

5 c) used to create a new or modified algorithm or program for use in said logic controller means; and

d) input to said logic-controller means from an off-site computer.

10 61. A method of alternating the cycles of redox potential and/or the predominant microbial respiration pathway within the contaminated geologic media, comprising the steps of:

15 a) introducing one or more of said chemical compositions of Claims 1, 6, 12, 17, 21, and 26 into the contaminated geologic media for one or more specified periods of time;

b) subsequently introducing one or more of said chemical compositions of Claims 1, 6, 12, 17, 21, and 26 into the contaminated geologic media for one or more additional specified periods of time; and

20 c) repeating and/or alternating steps a) and b) until the levels of contaminants in the contaminated geologic media have been reduced.

25 62. A method of alternating the cycles of redox potential and/or the predominant respiration pathways of MRP anaerobic microorganisms within the contaminated geologic media in-situ,

comprising the steps of:

5 a) performing a first phase of operation by adding one or more of the chemical compositions of Claims 1, 6, 17 and 21 into the contaminated geologic media for one or more specified periods of time at a specified delivery rate for promoting the growth of indigenous MRP anaerobic microorganisms via the denitrification pathway;

10 b) performing a second phase of operation by adding one or more of the chemical compositions of Claims 17, 21 and 26 into the contaminated geologic media for one or more specified periods of time at a specified delivery rate for promoting the growth of indigenous MRP anaerobic microorganisms via microbial respiration pathways involving manganese-reduction, iron-reduction and/or the reduction of other metals;

15 c) performing a third phase of operation involving the addition of the chemical composition of Claim 12 for one or more specified periods of time at a specified delivery rate for promoting the growth of anaerobic bacteria via the sulfate-reduction pathway; and

20 d) repeating and/or alternating steps a), b), and c) until the level of contaminants in the contaminated geologic media are reduced.

25 63. The method of Claim 61 whereby the specified number and duration of delivery events of the said chemical compositions promote the log-phase growth of the anaerobic biomass within the

contaminated geologic media.

64. The method of Claim 62 whereby the specified number and duration of delivery events of the said chemical compositions promote the log-phase growth of the anaerobic biomass within the contaminated geologic media.

65. The method of Claim 61 further including the step of introducing into the contaminated geologic media no chemical composition for one or more specified periods of time so as to allow for the decay of the anaerobic biomass within the contaminated geologic media.

66. The method of Claim 62 further including the step of introducing into the contaminated geologic media no chemical composition for one or more specified periods of time so as to allow for the decay of the anaerobic biomass within the contaminated geologic media.

67. The method of Claims 63 to 66 whereby the periods of log-phase growth and decay of the anaerobic biomass are cycled and/or repeated so as to optimize the biodegradation, transformation or detoxification of organic and/or inorganic contaminants by MRP anaerobic microorganisms.

68. The method of Claims 61 and 62, further including the step of introducing into the contaminated geologic media a carbon co-substrate selected from the group consisting of ethanol, methanol, or acetate for one or more specified periods of time at a specified delivery rate for promoting the biodegradation, transformation or detoxification of organic contaminants by MRP anaerobic microorganisms.

69. The method of Claims 61 and 62, further including the step of introducing into the contaminated geologic media a primary carbon substrate selected from the group consisting of ethanol, methanol, or acetate for one or more specified periods of time at a specified delivery rate for promoting the biodegradation, transformation or detoxification of inorganic contaminants by MRP anaerobic microorganisms.

70. Bioremediation apparatus including a vapor suppression system for reducing flash fire and/or explosion hazards in the contaminated geologic media comprising:

a) one or more gas cylinder tanks containing an inert gas for reducing oxygen gas ( $O_2$ ) concentrations within the contaminated geologic media;

b) control valve means connected to said one or more gas cylinder tanks;

c) pressure sensing means connected to said one or more gas cylinder tanks for providing a minimum pressure setting in which

said inert gas is discharged from said gas cylinder tanks to the contaminated geologic media; and

d) means for connecting a second well to said gas cylinder tanks

for supplying said inert gas to the contaminated geologic media.

71. Bioremediation apparatus in accordance with Claim 70, wherein said inert gas is argon, neon, krypton or xenon.

72. Bioremediation apparatus in accordance with Claim 70, wherein said control valve means is a manual shut-off valve.

73. Bioremediation apparatus in accordance with Claim 70, wherein said pressure sensing means include one or more in-line pressure gauges.

74. A method of suppressing malodorous compounds, comprising the steps of:

a) applying a chemical composition to an area containing malodorous compounds, such as hydrogen sulfide, to provide an electron acceptor compound to reduce the formation of hydrogen sulfide;

b) said chemical composition being selected from the group consisting of the chemical compositions defined above in Claims 1, 6, 17, 21, 26, 48, and 52.

## ABSTRACT OF THE DISCLOSURE

Liquid chemical compositions are disclosed for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in a contaminated geologic media under reducing conditions, including, but not limited to, denitrifying, manganese-reducing, iron-reducing and sulfate-reducing conditions. One such liquid chemical composition includes sodium nitrate in the range of one-fifth (0.2) to four (4) pounds per gallon of the chemical composition; sodium hexametaphosphate or other biologically hydrolyzable ring or linear polyphosphate in the range of one twentieth (0.05) to five (5) pounds per gallon of the chemical composition; a surfactant in the range of 0.01% to 10% by volume of the chemical composition; and a diluent in the form of water.

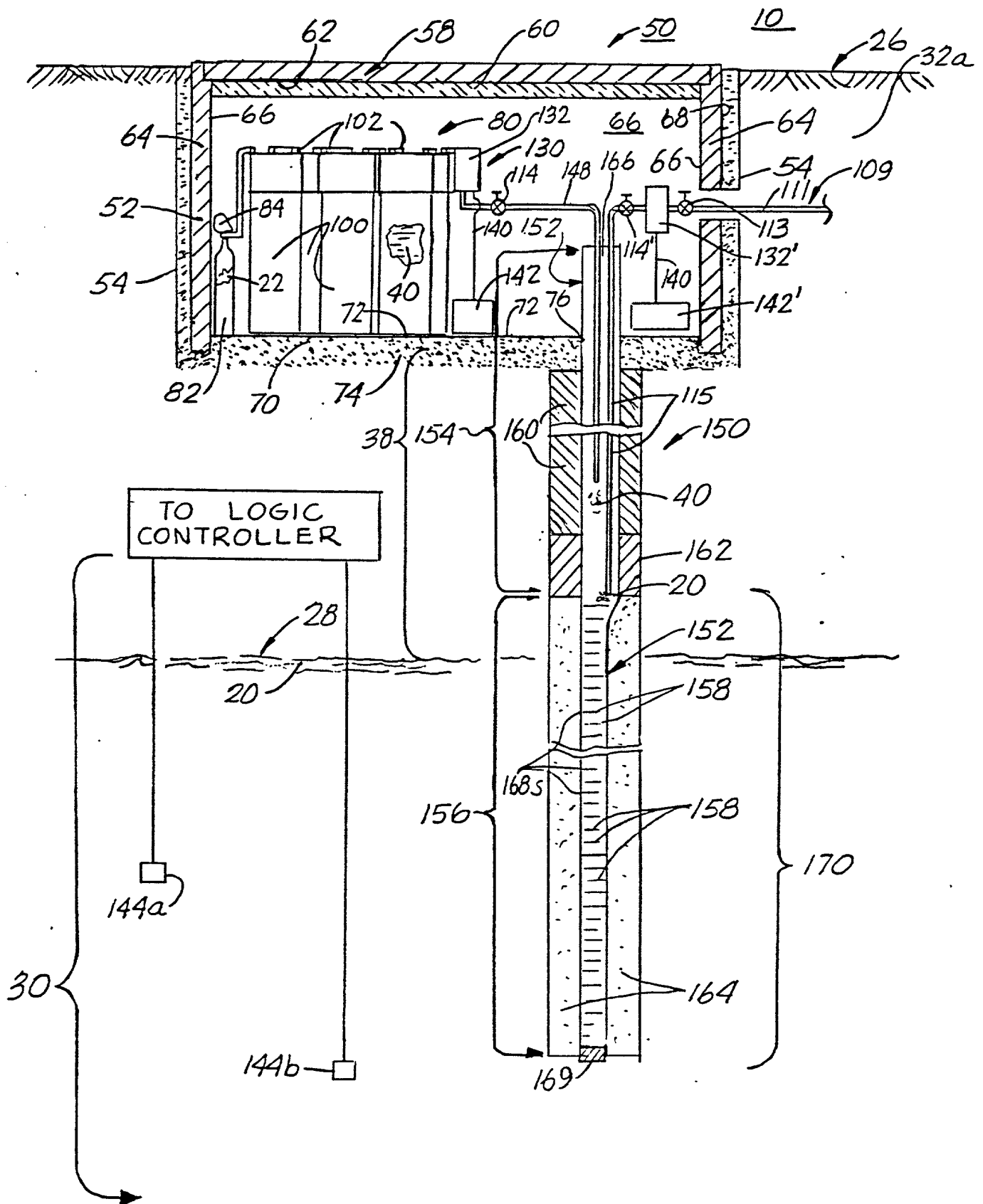
A bioremediation apparatus is disclosed for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in a contaminated geologic media. The bioremediation apparatus includes a first set of one or more storage tanks containing a chemical composition for anaerobic biodegradation of toxic compounds in contaminated geologic media; a plurality of quick disconnect valve couplings connected to the first set of storage tanks; at least one logic controller having a logic controller programmer component for opening and closing an automatic valve means connected to the first set of storage tanks to supply the chemical composition to the contaminated geologic media; and a screened well connected to the first set of storage

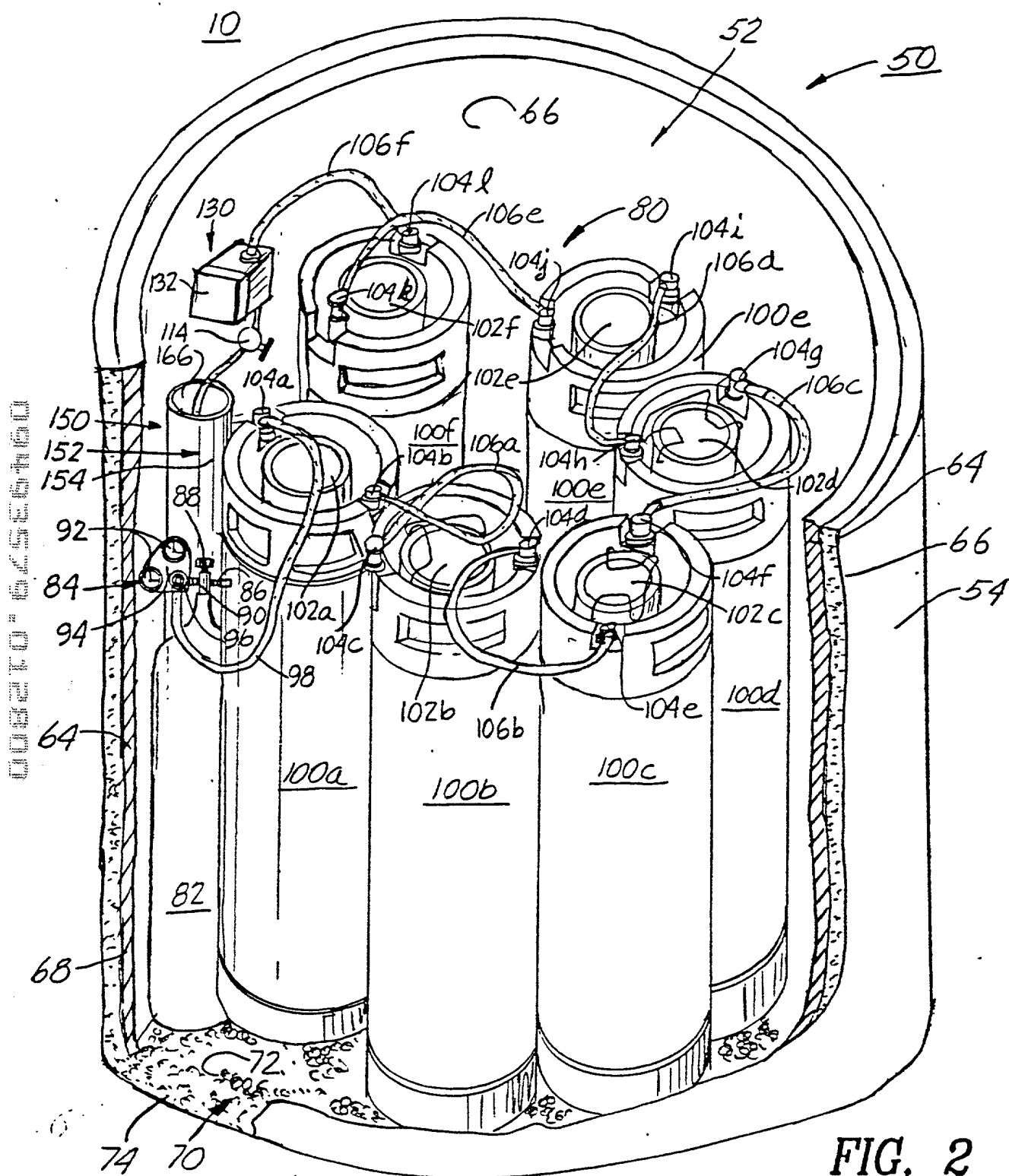
tanks for supplying the chemical composition to the contaminated geologic media.

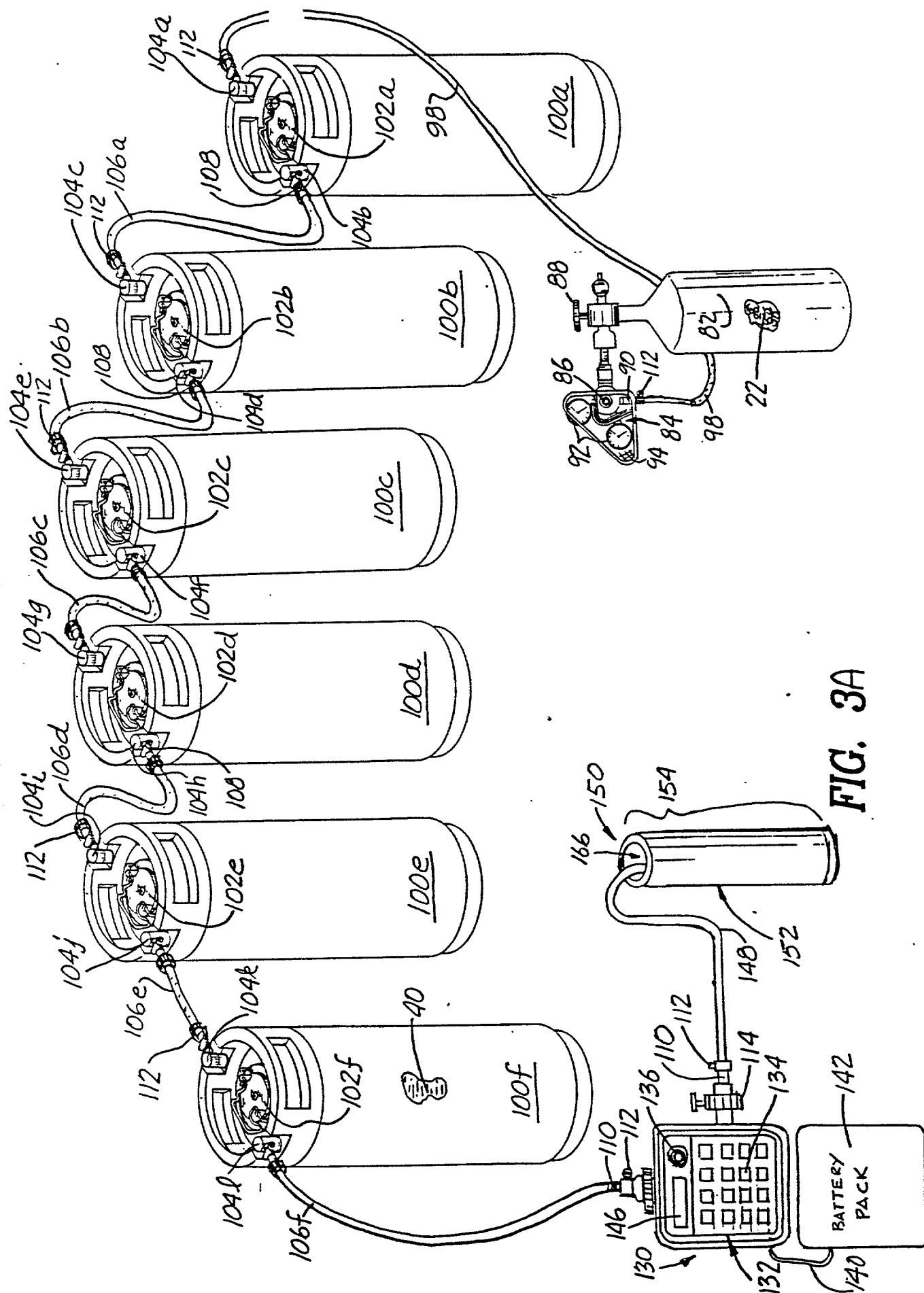
A method is disclosed for anaerobic biodegradation, detoxification, and transformation of toxic organic and inorganic compounds in a contaminated geologic media comprising the steps of pressurizing one or more storage tanks containing a chemical composition using an inert carrier gas; connecting a plurality of quick disconnect valve couplings to one or more pressurized storage tanks; connecting a well to an automatic ball valve for supplying the chemical composition and the inert carrier gas through the well to the contaminated geologic media; and opening and closing the automatic ball valve to dispense the chemical composition and the inert carrier gas under pressure through the well to the contaminated geologic media. Methods are also disclosed for alternating the cycles of redox potential and the predominant microbial respiration pathway within the contaminated geological media.



**Table 1**







**FIG. 3A**

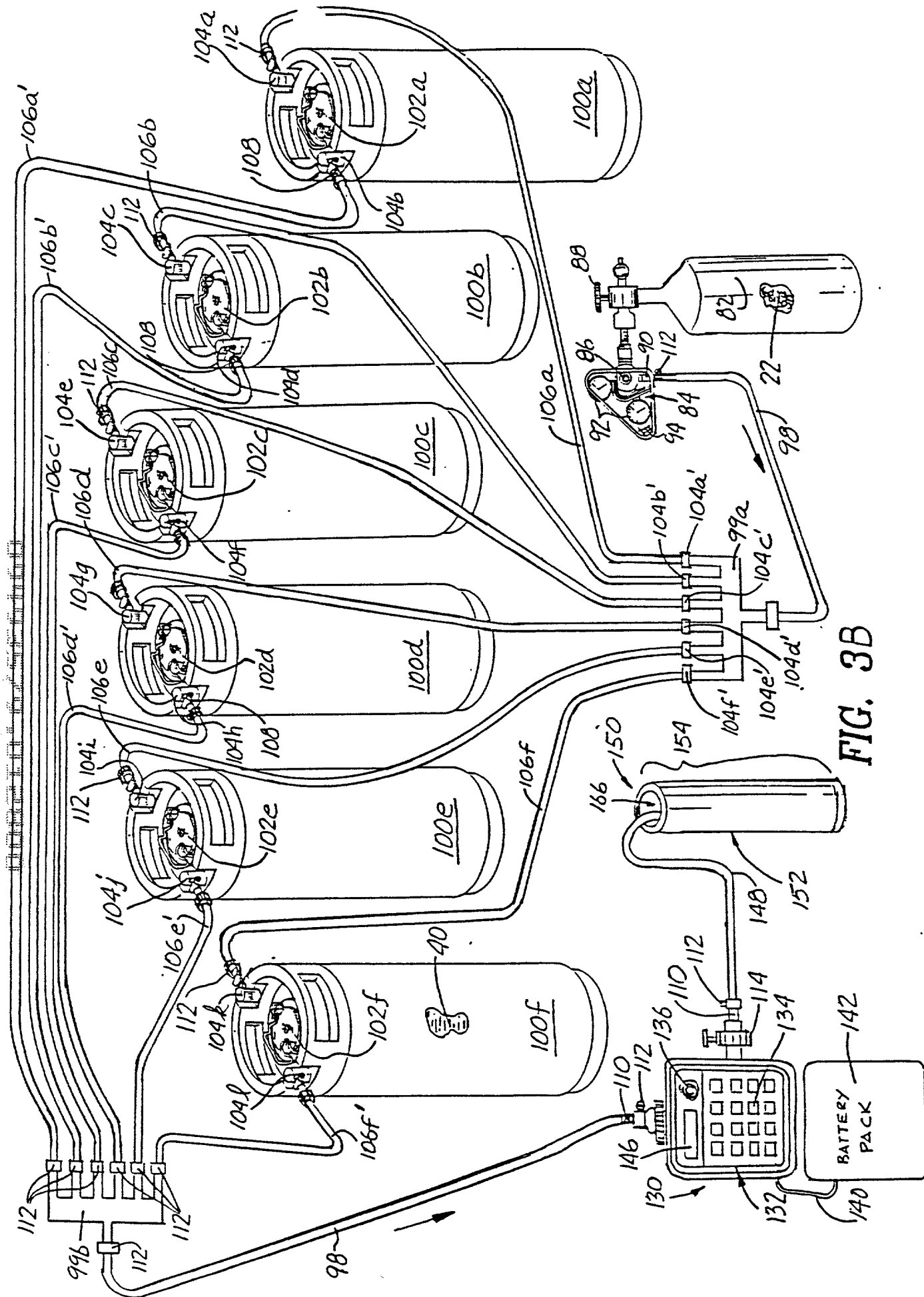
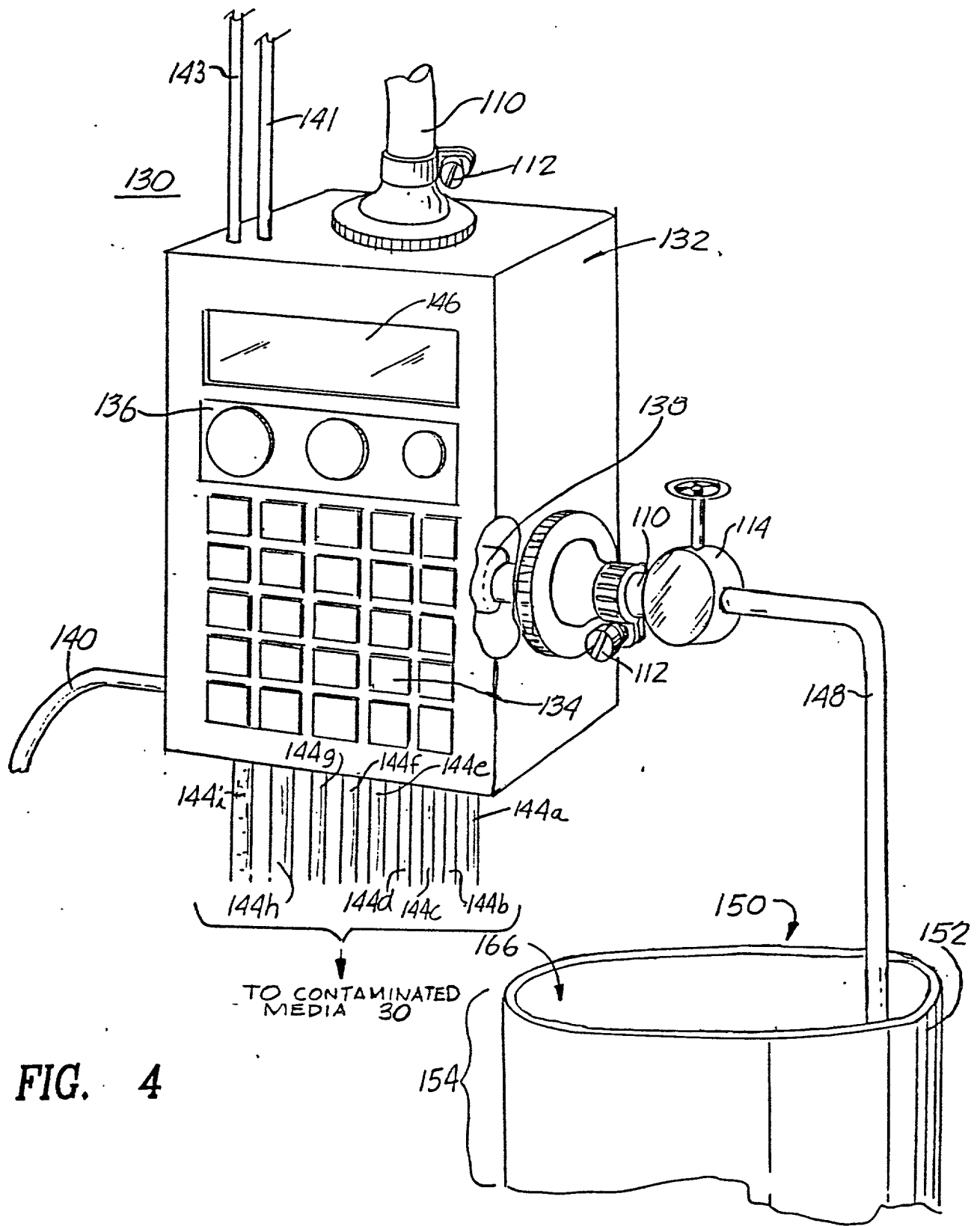


FIG. 3B



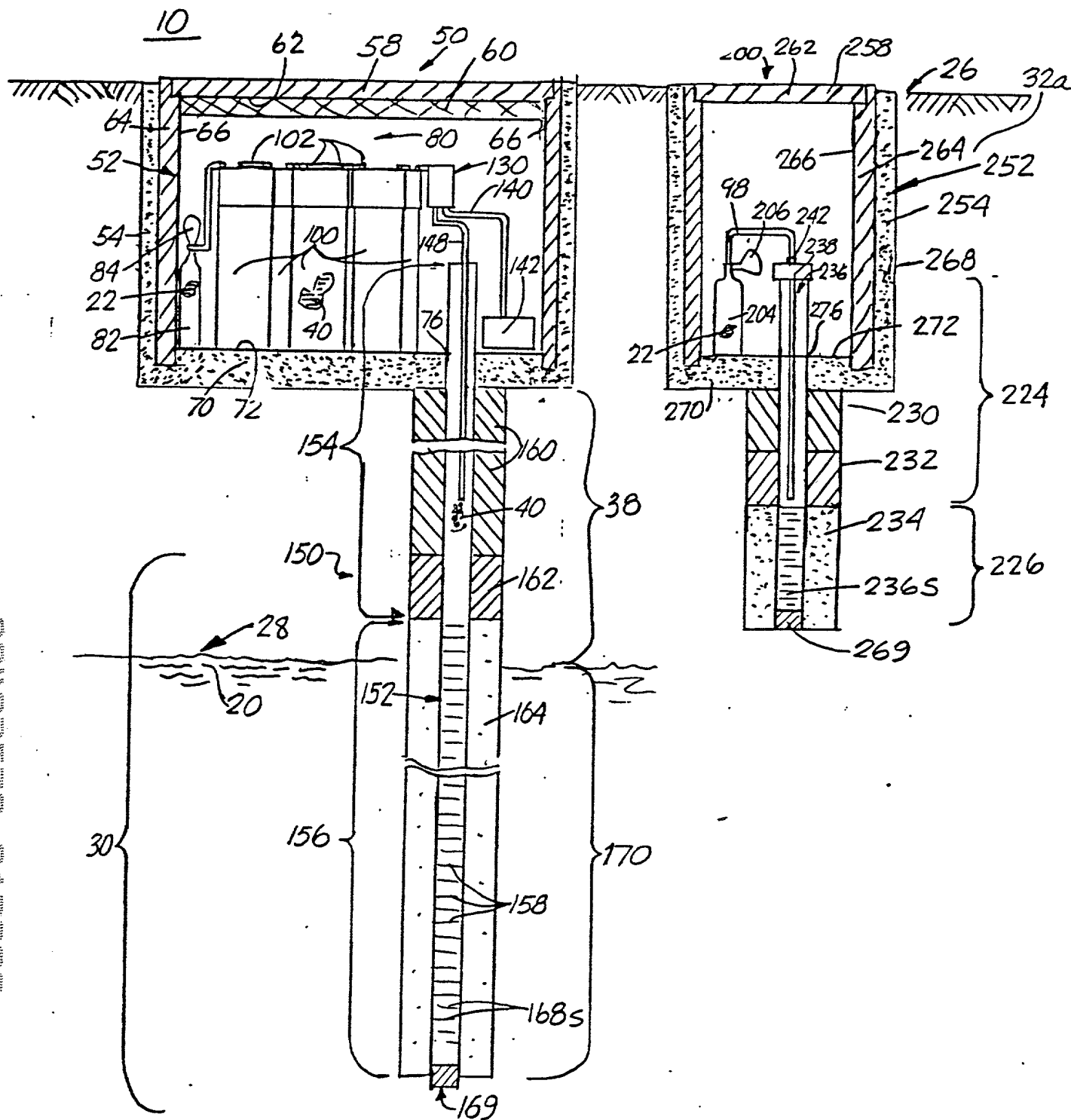


FIG. 5

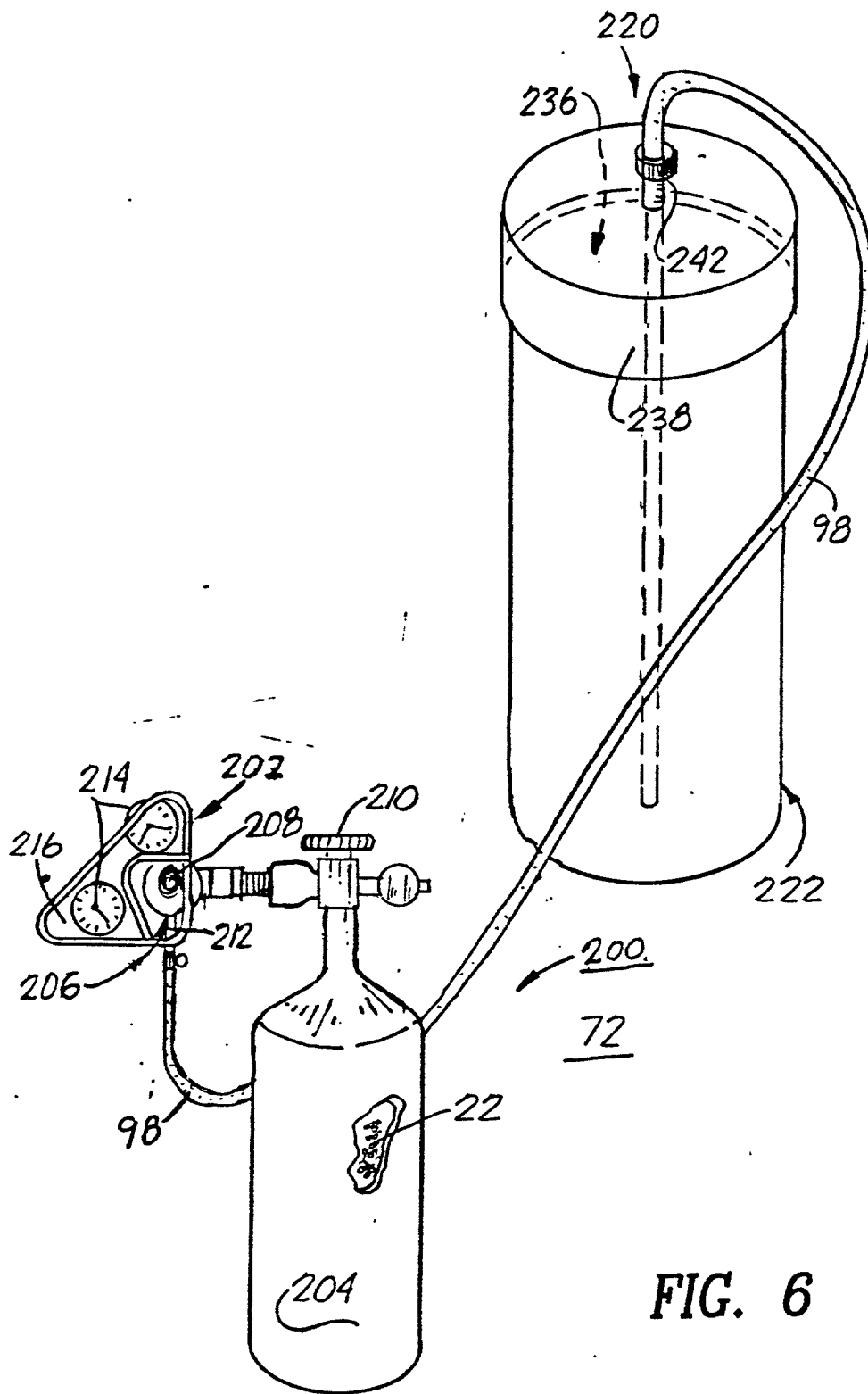


FIG. 6

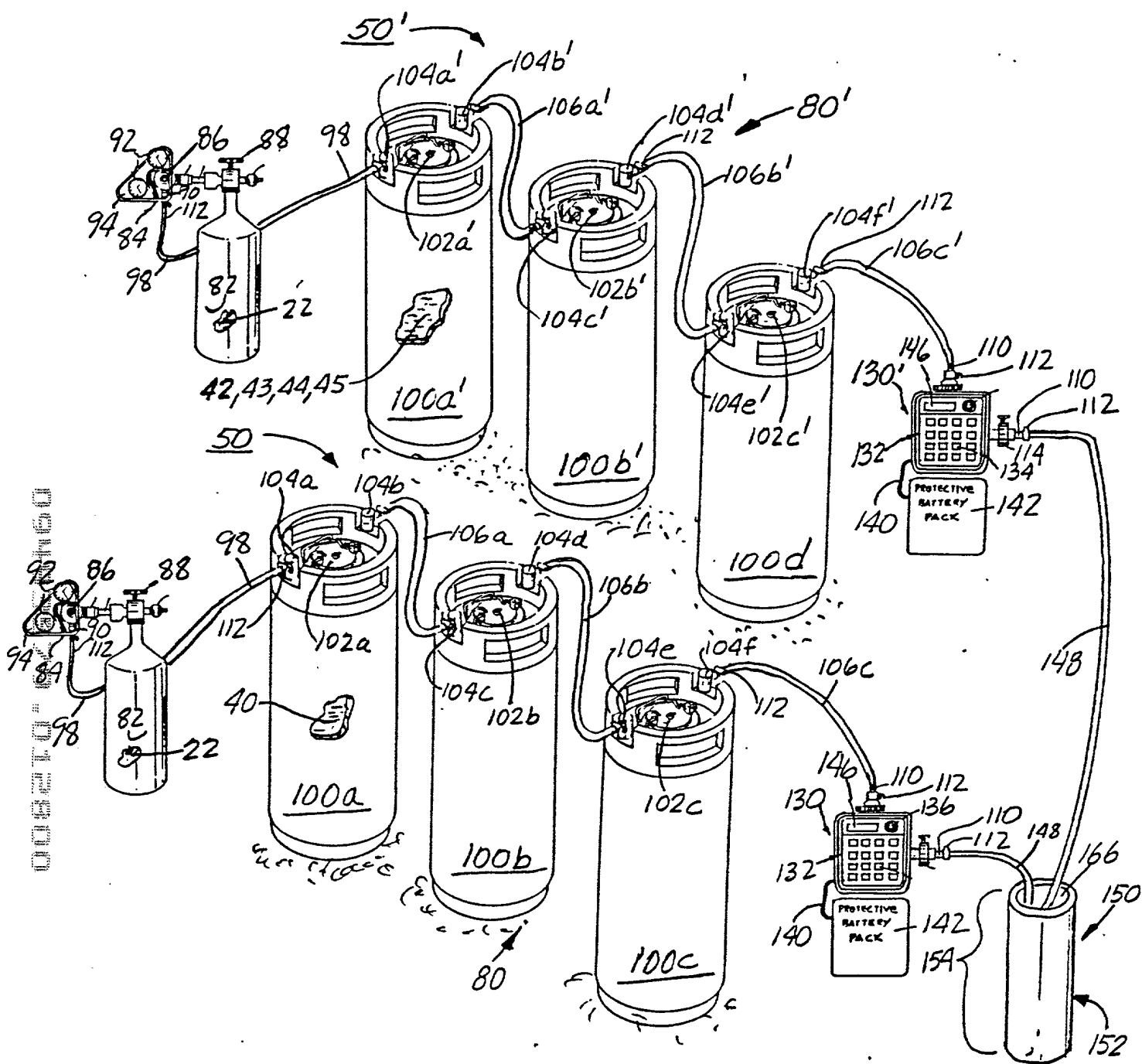
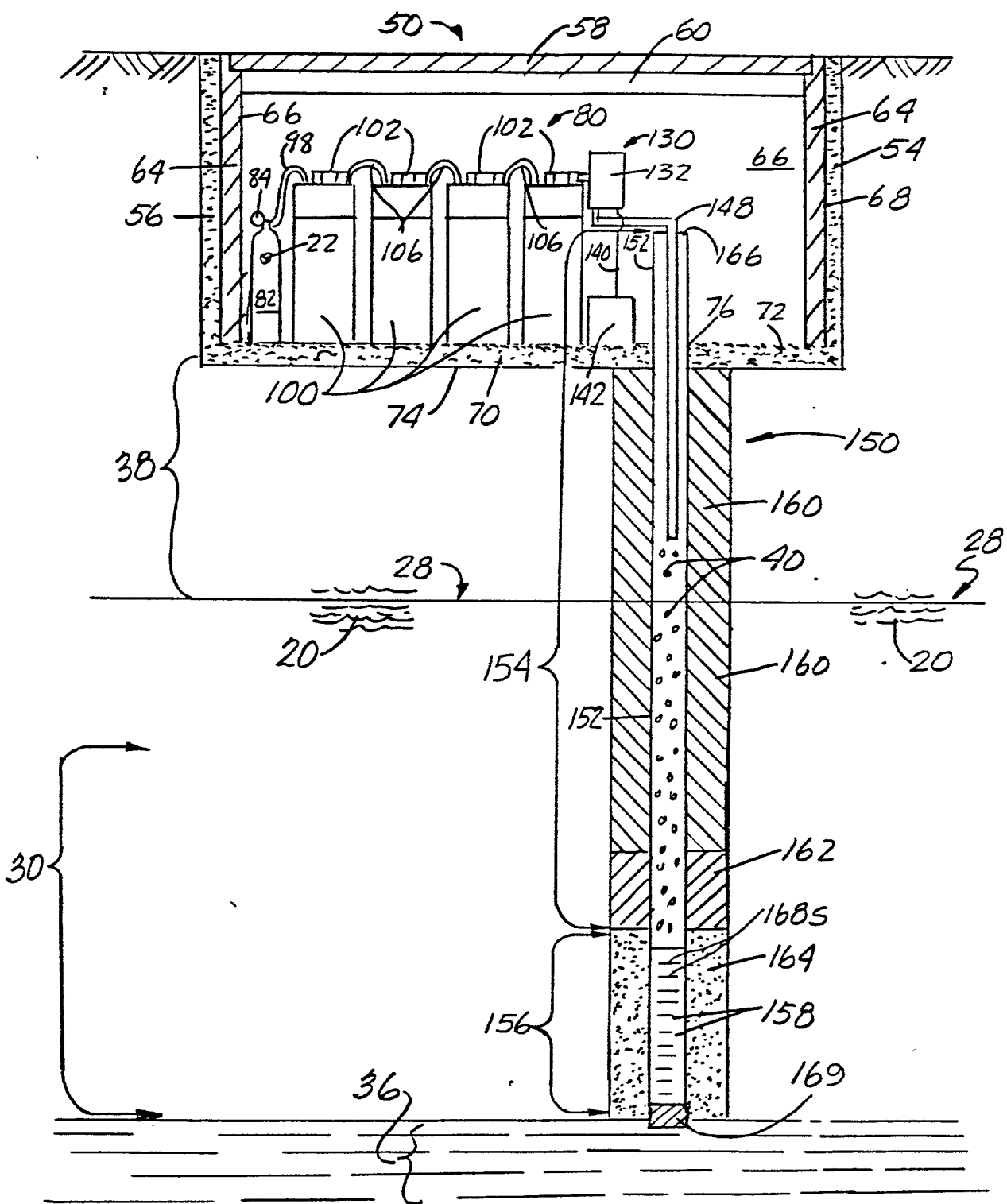


FIG. 7



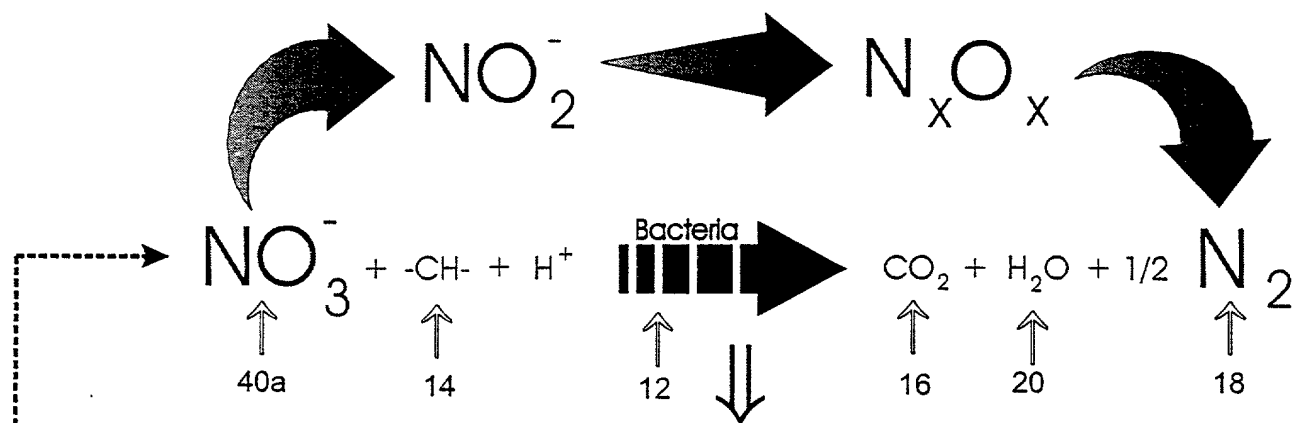


**FIG. 8**

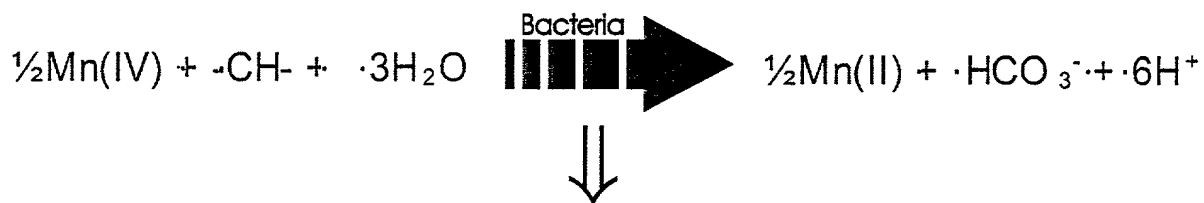
**FIG. 9**

10

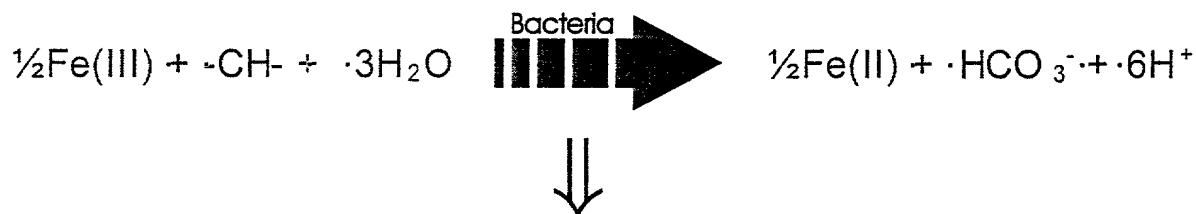
## Denitrification



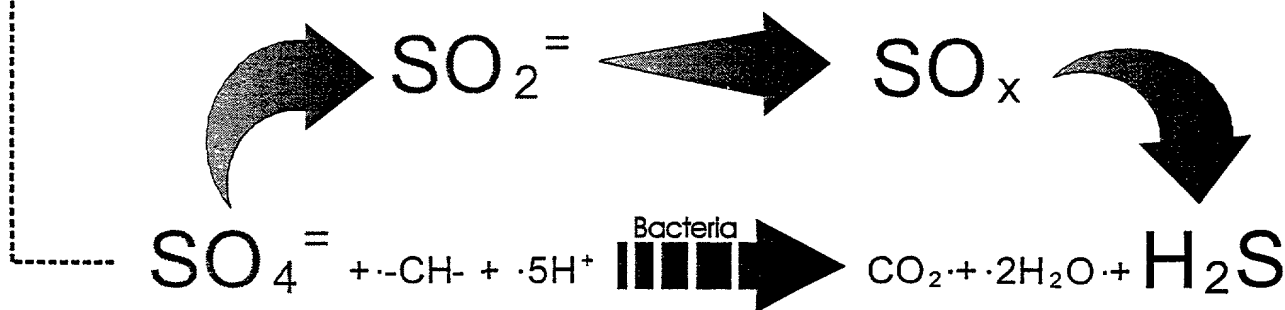
## Manganese(IV) Reduction



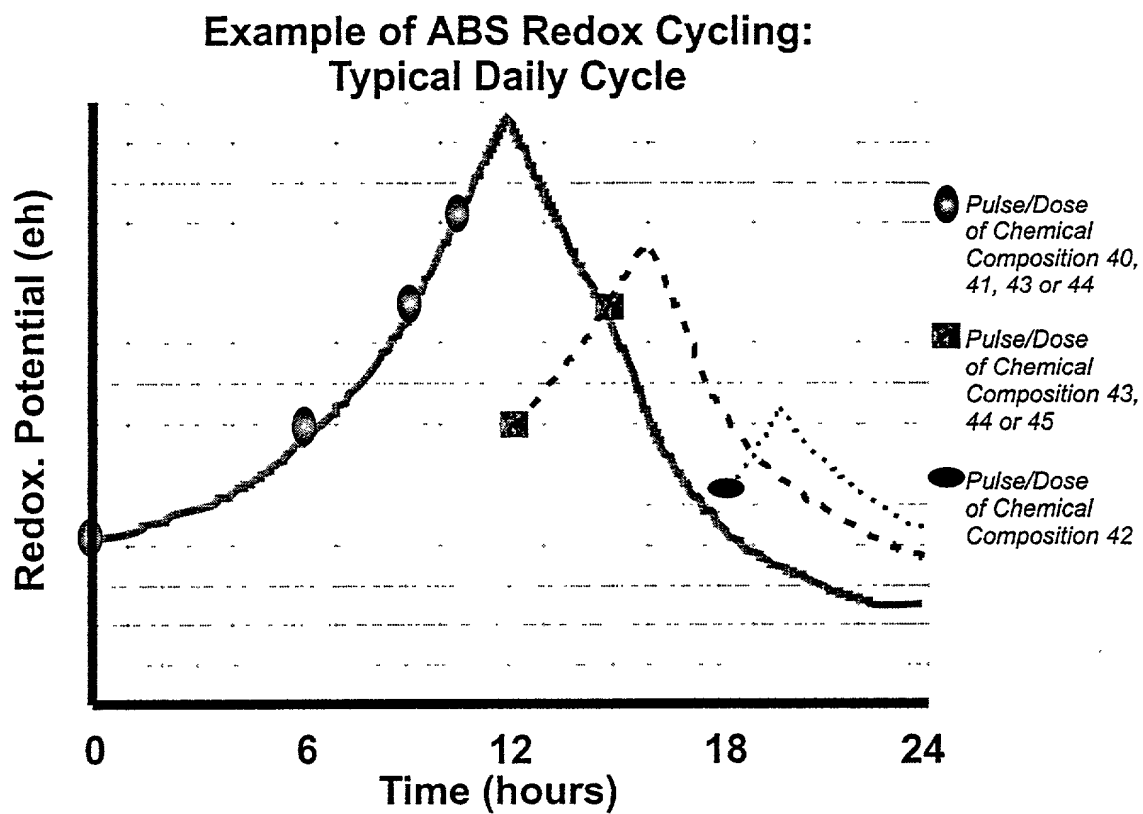
## Iron(III) Reduction



## Sulfate Reduction



**FIG. 10**



**FIG. 11**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled ANAEROBIC BIOREMEDIATION SYSTEM, the specification of which

(check one) ☒ is attached hereto.

☐ was filed on \_\_\_\_\_ as  
Application Serial No. \_\_\_\_\_  
and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s) Priority Claimed

(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
_____	_____	_____	_____	_____
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
_____	_____	_____	_____	_____
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
_____	_____	_____	_____	_____

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) \_\_\_\_\_ (Filing Date) \_\_\_\_\_ (Status—patented, pending, abandoned) \_\_\_\_\_

(Application Serial No.) \_\_\_\_\_ (Filing Date) \_\_\_\_\_ (Status—patented, pending, abandoned) \_\_\_\_\_

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Ezra Sutton, Reg. No. 25,770

Address all telephone calls to \_\_\_\_\_ at telephone no. (908) 634-3520

Address all correspondence to \_\_\_\_\_

EZRA SUTTON, P.A.

Plaza 9, 900 Route 9

Woodbridge, New Jersey 07095

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor ERIC CHRISTIAN HINCE

Inventor's signature [Signature] Date 5/22/97

Residence Harriman, New York 10926 Citizenship United States of America

Post Office Address 100 River Road, Triangle Plaza, Suite 8

Harriman, New York 10926

Full name of second joint inventor, if any Robert L. Zimmer

Second Inventor's signature [Signature] Date 5/22/97

Residence Harriman, New York 10926 Citizenship United States of America

Post Office Address 100 River Road, Triangle Plaza, Suite 8

Harriman, New York 10926

Full name of third inventor TIMOTHY H. ANDERSON

Inventor's signature X

Residence Harriman, New York 10926

Post Office Address 100 River Road, Triangle Plaza, Suite 8

Harriman, New York 10926

Citizenship United States of America

Date May 22, 1997

008210-0255460